NUTRIENTS IN GREAT SALT LAKE WETLANDS:

THE IMPORTANCE OF SEDIMENT FLUXES

AND BIOGEOCHEMISTRY

by

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ABSTRACT

The wetlands of Farmington Bay are ecologically and economically important to the surrounding Salt Lake City metropolitan area. This fresh water area is formed by the Jordan River and is thus subject to the river's water quality. Of chief concern in this study are contaminants that contribute to eutrophication such as ammonia, nitrate, and phosphate. The results of how sediments remediate nutrients can be included in previously created wetland health metrics.

Determining the factors that control if sediment acts as a sink or a source for ambient water nutrient concentrations could help influence surface water regulation. The sediment nutrient fluxes of nine wetland sites were measured for this study twice over the course of summer, 2014 by comparing the daily changes in nutrient concentrations of water with and without contact to the underlying sediment. These results were then correlated to biogeochemical parameters such as dissolved oxygen content, organic carbon availability, and size of bacterial community in the sediment for each site to explain which factors controlled sediment nutrient fluxes

Overall, in ambient conditions, most sites displayed sediment as an ammonia source during the daytime as decomposition likely outpaced nitrification. The sediment was also a sink for phosphate while nitrate trends varied between sites. After spiking the experimental chambers with nutrients to witness nutrient pulses, the nitrogen-cycling biological mechanisms were accelerated, while the sediment failed to absorb the extra phosphate. All but one site's sediment fluxes were statistically comparable via Z test, and the remaining

eight were used to conduct a principal component analysis to identify important variables. Ammonia sediment fluxes were found to be controlled by nitrifying bacteria and from the concentration gradient between the pore water and ambient water. Nitrate sediment flux correlated with the amount of denitrifying bacteria present in the sediment, the total solid organic carbon, and daily changes in temperature. Last, phosphate fluxes were influenced by diurnal cycling of the phosphate attached to iron in the sediment and changes in the dissolved oxygen saturation of the water column.

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NOMENCLATURE

ABS – Absorbance Value from Spectrometer

AOA – Ammonia-oxidizing Archaea

AOB – Ammonia-oxidizing Bacteria

AVG – Average Percent Error

DNA – Deoxyribonucleic Acid

DNRA – Dissimilatory Nitrite Reduction to Ammonia

DO – Dissolved Oxygen

FBWMA – Farmington Bay Wetland Management Area

GSL – Great Salt Lake

HAO – Hydroxylamine Oxidoreductase

IC – Ion Chromatograph

LFB – Laboratory Fortified Blank

LFM – Laboratory Fortified Matrix

LRB –Laboratory Reagent Blank

MMI – Multimetric Index

NOB – Nitrite-oxidizing Bacteria

NOR – Nitrite Oxidoreductase

NOS – Nitrite-oxidizing System

NPDOC – Nonpurgeable Dissolved Organic Carbon

NWI – National Wetland Inventory

PCA – Principal Component Analysis

PCR – Polymerase Chain Reaction

qPCR – Quantitative Polymerase Chain Reaction

QA/QC – Quality Assurance/Quality Control

rRNA – Ribosomal Ribonucleic Acid

SAV – Submerged Aquatic Vegetation

SED – Sediment Column

STD – Standard Deviation

TF – Terminal Fragment

TIN – Total Inorganic Nitrogen

TOC – Total Organic Carbon

TRFLP – Terminal Restriction Fragment Length Polymorphism

TS – Total Solids

UDWQ – Utah Division of Water Quality

UDL – Under Detection Limits

USEPA – United States Environmental Protection Agency

VS – Volatile Solids

WC – Water Column

WQ – Water Quality

WWTP – Wastewater Treatment Plant

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CHAPTER 1

INTRODUCTION AND OBJECTIVE

Introduction

On Wetlands

Wetlands have recently gained notice as one of the most economically beneficial terrestrial ecosystems on Earth (Costanza et al., 1997). Since a large variety of wetlands exist, the term is most accurately a catch-all for terrestrial environments with continuous standing water and saturated soil (Finlayson et al., 1995). Lakes, rivers, floodplains, and even coral reefs can be considered wetlands by this definition, while the chief concern of this study is natural and man-made marsh wetlands in Utah, USA (*Wetlands International*, 2015). These wetlands can be considered riparian as most of the surrounding landscape is arid. Wetlands serve a dual purpose of acting as both a flood buffer for cities and as a refuge for a large diversity of species, as is the case for the Great Salt Lake Wetlands. The Great Salt Lake Wetlands, which remain fresh due to the influent from streams, runoff, and the Jordan River, act as an important ecological area both to migratory birds and native species, but there are hidden benefits—these wetlands also act as a sort of filter to remove contaminants from surface water (Carling et al., 2013; *Development of an Assessment Framework*, 2009). However, portions of the Great Salt Lake wetlands have fallen victim to disturbances such as invasive species (*Phragmites australis*) and eutrophication (Carling et al., 2013). These dual ecological disturbances could cause ecological collapse in this fragile wetland ecosystem.

The Multimetric Index

Recently, efforts have been made to better understand the complex nature of these wetlands in order to preserve them and their place in this region's ecology; important efforts include creating a way to decide which local wetlands would benefit most from management strategies. Namely, a multimetric index (MMI) is being developed by the Utah Division of Water Quality (UDWQ) to help rate the impairment of the wetlands surrounding the Great Salt Lake (*Development of an Assessment Framework,* 2009). The MMI focuses on factors such as water chemistry, submerged aquatic vegetation (SAV) cover, and the abundancy and species of invertebrates in the wetland (*Development of an Assessment Framework*, 2009). Little information has been gathered about the status of the Great Salt Lake wetlands with regards to specific nutrient fluxes and the microbial mediation of these fluxes.

Biogeochemistry and Importance

Biogeochemistry is a broad subject which unifies the chemical, physical, geological, and biological processes of the environment. For example, modelling the spatial cycling of nitrogen and phosphorus in wetlands requires advanced biogeochemistry concepts (Schlesinger, 2005). Understanding the origins and destination of nitrogen and phosphorus in the wetlands is crucial as it has been found that enhanced nutrient concentrations could be the cause of eutrophication and the prevalence of the invasive species *Phragmites australis* in the wetlands (Carling et al., 2013). Since water chemistry, but not sediment biogeochemistry, has already been chosen as a part of the original MMI, sediment biogeochemistry of the wetlands is the focus of this study (*Development of an Assessment Framework*, 2009). Hopefully, it can be proven that the biogeochemistry of sediment can be just as much of a factor to water quality as the other MMI criteria.

Incorporation of Sediment Health into MMI

Well-functioning sediment allows for uptake of nutrients at a far greater rate than surface water by providing a medium in which nutrient-absorbing SAV can grow, being the main stage for denitrification, and by simply being a site for absorption of nutrients in the wetlands (Carling et al., 2013; Kadlec, 1999). However, in order to include a whole new factor into such a robust analysis as the MMI, more information is needed on which exact processes in the sediment control its nutrient cycling capability, and how these processes vary with regard to sediment health. Afterwards, reference "well-functioning" wetlands would need to be analyzed based on the factors which are identified to mediate sediment flux. But first the physical, chemical, and bacterial processes that control the wetland sediment flux of nitrogen and phosphorus need to be analyzed.

Justification of Objectives

Based on previous studies regarding nitrogen sediment dynamics, it seems that bacterial processes control more nitrogen transfer than physical or chemical processes. Studies show that nitrogen stored in vegetation biomass is the least active pool of nitrogen in sediment, and bacterial processes allow nitrogen to become bioavailable, partially through ammonification (Reddy et al., 2008). In addition, the only way to completely remove nitrogen from a wetland body (other than advection that simply moves nitrogen) is denitrification – a widespread bacterial process (Smith et al., 2009). To make the connection between nitrogen-cycling bacteria and sediment nutrient cycling, the nitrogen-cycling processes should be quantified and compared to sediment flux using statistical analysis. Phosphorus cycling is dictated by both biotic processes, such as photosynthesis, and abiotic processes, such as adsorption. Both transformations are interconnected through diurnal

cycling of oxygen concentrations - which are more important? (Reddy et al., 1999)

While chlorophyll-a in the water column will be measured, the algae and photosynthetic organisms on the sediment surface will be neglected, as the purpose of the experiment is to isolate sediment interactions. Therefore, with much of the biotic processes incorporated in phosphorus cycling removed from the analysis, the focus will be on abiotic phosphorus cycling. Determining the amounts of phosphorus attached to different minerals in the sediment could give a view into which abiotic processes control phosphorus sediment flux. To better understand the sediment biogeochemistry's role in wetland nutrient cycling, the following objectives were created.

Objectives

This study has the following distinct objectives.

- Evaluate nutrient fluxes between the sediments and the water column in Farmington Bay wetlands.
- Evaluate the role of nitrification and denitrification biological processes in the N cycle in sediments.
- Evaluate different forms of phosphorus by conducting sequential P speciation.

The listed objectives of this study should answer many questions, such as which factors are the most important in sediment nutrient cycling? Also, how large of a role do sediments play in the wetland's nitrogen cycle? What is the sediment's nitrification and denitrification rate and are these transformation routes dependent on bacterial count or the species of bacteria present? Last, can the sediment be used as a reliable sink for phosphorus? Based on the literature review for this study, the following hypotheses were created:

1. A gauge of sediment bacterial processes can be used to assess wetland health with regard to nitrogen cycling.
2. Phosphorus cycling occurs as a redox reaction to abiotic conditions such as oxygen content and temperature of ambient water.
3. The Great Salt Lake wetlands have potential to be used as a means of nutrient remediation.

To check these hypotheses and achieve the objectives of this study, the following tasks were created.

- Task 1: Design and fabricate in situ flux chambers and conduct flux measurements.
- Task 2: Measure P speciation using a sequential extraction technique and measure other parameters in sediment core samples.
- Task 3: Determine the rates of nitrification and denitrification in sediment using serum bottle tests.
- Task 4: Identify the bacteria participating in nitrification and denitrification using advanced molecular tools.
- Task 5: Data analysis using statistical software "R".

CHAPTER 2

RATIONALE AND BACKGROUND INFORMATION

General Wetland Information

Wetland areas are an important natural resource that should be protected due to the inherent ecological, societal, and environmental benefits. It has been proven that wetland areas can be used as a sink for carbon—as long as the amount of carbon dioxide absorbed by wetland areas outweigh the amount of methane produced by basis of greenhouse gas release, wetlands can actually become a net carbon sink (Whiting et al., 2001). Wetlands are also important flood control areas as they can collect surplus rainfall or snowmelt (*Wetlands: Protecting Life and Property from Flooding*, 2006). Even smaller wetland areas can collect a large amount of excess water if they are in close proximity to the water source. Therefore, wetlands can help prevent costly property damage from flooding. In addition to these other benefits, wetlands can serve as giant remediation ponds (Hantush et al., 2013). Due to the high retention time of water in wetlands, excess nutrients and contaminants in wetland waters can be removed via the natural processes of nitrification or denitrification, which metabolize nitrogen (Hantush et al., 2013). On the other hand, wetlands use this high retention time for cycling between organic/inorganic and soluble/insoluble phases of phosphate rather than for net release (Reddy et al., 1999). Because of these benefits, and others, a study by economists in 1997 actually quantified wetlands as the most monetarily valuable terrestrial ecosystem per acre as a sunken cost estimate (Costanza et al., 1997).

This type of ecosystem makes up 6% of the biosphere on Earth, however, the biogeochemistry remains poorly understood due to the complex variety of processes taking place (Reddy et al., 2008). Many different varieties of wetlands exist in the world, differing in their water content, flow regime, and location in a surrounding region, among other factors (Finlayson et al., 1995). Much attention has been paid to how different types of wetlands function. As a result, a national wetland inventory (NWI) has been created to register the exact locations of all wetlands in the US by type (*Alternative Futures Analysis*, 2010).

The many types of wetlands are classified primarily by the biome in which the wetland is located. For example, wetlands can behave differently in coastal settings versus alpine settings and thus differ in their classification (Central European University, 2015). Also, wetlands can vary based on the sediments which the surface water is exposed to—both organic- and mineral-dominated soils have different interactions in wetlands (Central European University, 2015). Of focus for this project are the wetlands which are found in semiarid regions such as Utah. The plant productivity of desert wetlands typically outpaces the productivity of upland regions; therefore, desert wetlands are important to agriculture in a region typically lacking moisture (*Chapter 4 - Wetlands*, 2014).

Two important types of wetlands are dictated by the flow regime of the water in them: impounded wetlands and riparian wetlands. Impounded wetlands are man-made as a result of the construction of dikes, berms, ditches, and culverts, while riparian wetlands occur naturally where land comes in contact with streams or rivers (*Chapter 4 - Wetlands*, 2014). Riparian wetlands occur when influent flows meet the Great Salt Lake, for example, while impounded wetlands occur wherever people design them to be built.

Other wetlands that can be found in the Great Salt Lake Wetlands include playas and

emergent wetlands (*Alternative Futures Analysis*, 2010). Playas are shallow, temporary pools which are subject to seasonal changes and evaporation, while emergent wetlands contain emergent plants, which root in the bottom of the wetlands while the rest of the plant extends out of water (Aber, 2015). The main differences between emergent and impounded wetlands are hydrological—naturally occurring emergent wetlands fluctuate in depth based on flow conditions, while artificial impounded wetlands maintain the same depth unless changed by operators in a flow-regulated wetland (Gopal, 1999). Typically, emergent wetlands tend to support more diverse aquatic populations than impounded wetlands and already contain the rich bacterial communities needed for nutrient remediation (Gopal, 1999). Impounded wetlands do not contain the same bacterial communities as emergent wetlands from the onset (Gopal, 1999). Because impounded wetlands occur unnaturally, the nutrient mechanisms of these wetlands do not always behave properly, so the wetland's health must be monitored. On the other hand, terrestrial animal and bird populations tend to react positively to the construction of wetlands (Strand et al., 2013). With regard to nutrient remediation, not much is known about which wetlands would be of most value in the long run (Gopal, 1999).

Great Salt Lake Wetlands

The Great Salt Lake contains an area just under 5100 km^2 that receives industrial, urban, mining, and agricultural discharge from more than 1.7 million people (Naftz et al., 2008). The lake is surrounded on the east by freshwater tributaries that replenish the Great Salt Lake with fresh water collected from the nearby rivers and also from snow melt. These tributaries flow into the Great Salt Lake Wetlands, which comprise 427,000 acres (*Chapter 4 - Wetlands*, 2014). Farmington Bay Wetlands, the main region of focus for this study,

encompass roughly a third of the Great Salt Lake wetlands (Hoven, 2010). In addition, roughly 100,000 acres of the Great Salt Lake Wetlands are classified as impounded and are managed by State and Federal agencies and regional hunting clubs. The rest of the wetlands are emergent or riparian and not managed by protection agencies or hunting clubs (*Chapter 4 - Wetlands*, 2014).

The Great Salt Lake wetlands have gained attention lately due to the identification of high ambient nutrient concentrations as being a cause of several issues in the wetlands. Excess surface mat growths as a result of high nutrient concentrations have been negatively impacting SAV health by blocking sunlight (Carling et al., 2013). In addition, invasive species such as phragmites (*Phragmites australis*) are spreading partially as a result of higher nutrient concentrations (Carling et al., 2013). While specific factors controlling the internal cycling of nutrients in sediments are still being understood, the statuses of the inlet streams into the wetlands are easily understood.

The Farmington Bay Wetlands are a highly modified wetland system, as every stream draining into the wetlands has been altered into a system of canals and drainage ditches for urban and agricultural uses (*Alternative Futures Analysis*, 2010). The main types of wetlands present are playa, emergent, impounded, and riparian (*Alternative Futures Analysis*, 2010). Playas were not investigated in this study due to the very shallow (if at all present) water depth, and the majority of the wetlands classified as fringe wetlands directly border Farmington Bay; therefore, emergent and impounded wetlands were the main focus of this study (*Alternative Futures Analysis*, 2010).

Since the Jordan River flows into the Farmington Bay Wetlands from the south, most of the fresh water entering Farmington Bay in this region is subject to the water quality of this river which travels through the Salt Lake Valley. However, the Jordan River receives

water from industrial, urban, mining, and agricultural discharge from the surrounding watershed, which may degrade the river's water quality (Naftz et al., 2008). Half of the Jordan River flow is diverted to the State Canal before it reaches the southern portion of the Farmington Bay Wetlands (*Alternative Futures Analysis*, 2010). The Farmington Bay wetlands also receive water from the Wasatch Range on the east side. The Baird Creek, Kays Creek, and Holmes Creek tributaries originate in the Wasatch Range and collect runoff and effluent from Davis County downstream before joining the wetlands (*Alternative Futures Analysis*, 2010). While there are current discharge limits on the amount of nitrogen and phosphorus that can be in secondary effluents, the expected population increase of Salt Lake City and surrounding areas suggests that more nutrients could end up in the Farmington Bay Wetlands via the Jordan River/State Canal and other water inputs, such as nonpoint sources. Population increases correspond to infrastructural changes to wastewater treatment plants (WWTPs), as more wastewater will need to be treated—this could result in potentially higher costs for waste treatment, or risk the consequences of higher nutrient concentrations in the Jordan River. Higher concentrations of nitrogen or phosphorus in surface water can have dire consequences overall.

Multimetric Index Background

Nitrogen, Phosphorus, and Wetland Health

Nitrogen and phosphorus, required by all organisms to survive, are often limiting growth factors for most autotrophs (Elser et al., 2007). Although these nutrients are building blocks for protein, enzymes, and other cell material, their excess quantities can cause unregulated growth (Elser et al., 2007). These increased nutrients can cause many problems such as eutrophication. This is the case with the Farmington Bay wetlands, namely surface

mat growth and the prevalence of the invasive species phragmites (Carling et al., 2013). Primary producers are changing from seagrass to microalgae as a continuous oversupply of nutrients from various sources keeps the algae alive and thriving until the algae die and cause a buildup of detritus (Engelsen et al., 2008).

The amount of nitrogen and phosphorus flowing into the Farmington Bay Wetlands may not be an issue if the wetlands display a certain level of healthy characteristics and the ability to degrade/assimilate these concentrations. Healthy wetlands can be categorized as those which encourage species diversity through maintenance of the natural food chain and fulfill the environmental benefits of wetlands such as nutrient remediation and flood prevention (*Development of an Assessment Framework*, 2009). To help gauge the health of wetlands, a multimetric index (MMI) is being developed by the Utah Division of Water Quality (UDWQ). The MMI aims to convert multiple wetland quality variables, measurable on and off site, into a single health value on which wetlands can be scored (*Development of an Assessment Framework*, 2009). This serves a dual purpose of setting a baseline example for healthy wetlands and simplifying the difficult objective of designating which wetlands are potentially degraded and most appropriate for regulation strategies (*Development of an Assessment Framework*, 2009). Work has been made to start creating the wetland MMI and one objective of this project is to provide evidence for the importance of adding sediment data into the MMI.

Previous wetland health indicators relied heavily on the DO concentrations and pH of surface waters in wetlands (*Development of an Assessment Framework*, 2009). However, due to the inconsistencies of DO concentrations and pH from high diel changes, the UDWQ was prompted to develop a more comprehensive system for analyzing wetland health (*Development of an Assessment Framework*, 2009). As a response, UDWQ has created a

preliminary multimetric index as a way to gauge wetland health. In this multimetric index, the preexisting wetland quality indicators of pH and DO are expanded on. In addition, a variety of different health indicators are also scored, and the sum of these scores results in an overall multimetric index (*Development of an Assessment Framework*, 2009).

The health indicators used in this preliminary MMI are the condition of a wetland's water chemistry, submerged aquatic vegetation, surface mat, and benthic macroinvertebrates (*Development of an Assessment Framework*, 2009). These factors were chosen to gauge the ecological health of wetlands, as ecosystems which maximize the amount of biomass and symbiotic interaction between organisms per amount of energy put in the system benefit the environment the most (Odum,1969). For example, nutrients are exchanged, not released, more so in these idealized ecosystems than in impaired ecosystems (Odum, 1969). While these previously identified health indicators are a great gauge of the factors influencing optimum ecological succession of a wetland, it does not give much information on the nutrient trends which cause the deterioration of wetland health. For example, state measurements of ambient nitrogen, phosphorus, DO, chlorophyll-a, and total suspended solids are considered for the wetland chemistry portion of UDWQ's multimetric index—but these cannot predict the overall trend of nutrients in the wetlands (*Development of an Assessment Framework*, 2009). While SAV and photosynthetic organisms can contribute to the fate of nitrogen and phosphorus in the wetlands, the sediment must also be considered in order to help explain the overall trend of nutrients in order to enhance the MMI. The multimetric index report acknowledges that the sediment is a readily available supply of phosphorus, and many of the processes involved in nitrogen removal are involved in the sediment (*Development of an Assessment Framework*, 2009). Even if only justified by the large surface area alone, sediment functionality should be included as a health indicator of

wetlands. If the nutrient cycling capability of a wetland's sediment is considered along with the previously identified "state point" health indicators such as wetland chemistry and SAV cover, a long term sustainability model could be derived from the MMI by predicting how the sediment will mediate nutrient cycling. Therefore, much needs to be learned about the nutrient dynamics of the Farmington Bay wetlands in order to predict how the wetlands will respond to the extra nutrients from the growth of Salt Lake City. More specifically, the nitrogen and phosphorus cycles in the sediment are of most concern when studying the health of wetlands.

Sediment Contributions to Nutrient Cycling

Before nitrogen and phosphorus cycles are covered in depth, the importance of sediment in biological cycles in the wetlands should be understood. Sediments are an important medium for nutrient cycling in wetlands, particularly as the site of organic matter accumulation in the wetlands (Kadlec et. al, 2009). Processes which occur with and without exposure to oxygen can take place simultaneously in sediment at different depths from the surface and as a result of dissolved oxygen changes from photosynthesis (Kadlec et. al, 2009). Processes such as sedimentation and decomposition ensure that the sediments remain rich in inorganic nitrogen, while bacterial processes and interactions with both plant and animal life remove nitrogen from the water (Reddy et al., 2008; Wetzel, 2008). Sediment acts as a great medium for plant and bacterial growth. Previous UDWQ efforts have confirmed the importance of sediments to overall plant health in wetlands (Carling et al., 2013). Sediment can also, in fact, be used as a filter for the water column, in a sense as the paired anoxic and aerobic zones in the sediment can facilitate microbial nitrogen cycling to remove nitrogen nutrient contaminants from the water column (Kadlec et. al, 2009). The importance

of wetland sediments in the bioremediation of fossil fuels has already been covered in depth by the EPA; a guideline for the use of wetlands in bioremediation was created as early as 2001 (Zhu et al., 2001). Finally, the biological complexity of wetland sediments far outstrips what can be constructed in a WWTP (Brix, 1993). For example, microorganisms located in the sediment, or attached to macrophytes in the sediment, are responsible for metabolizing soluble organic compounds and removing nitrogen through nitrification-denitrification (Brix, 1993).

In addition to these other benefits, sediment plays an important role in all nutrient cycling. The sediment layer is the main region in the wetlands that can facilitate nitrogen transformation steps that require anoxic conditions such as denitrification (Smith et al., 2009). The sediment acts as either a source or sink of nutrients based on a variety of physical, chemical, and biological conditions, some of which are investigated during the course of this study (Reddy et al., 2008). Physically, the sediment supplies absorptive properties that can trap or release nutrients. Chemical properties of the water column such as pH and dissolved oxygen are also regulated by the sediment (Wetzel, 2008). The sheer surface area of sediment in contact with surface water - 0.05 km^2 to 6.05 km^2 - makes understanding nutrient fluxes very important.

Nitrogen Cycling

Nitrogen Overview

The nitrogen cycle is an interconnected process between land organisms, bacteria in sediment, chemical reactions, and weather contributions, as shown in Figure 2.1 (Brown et al., 1991). All components of Figure 2.1 (with the exception of ammonia, nitrate, and nitrite) can be considered organic nitrogen.

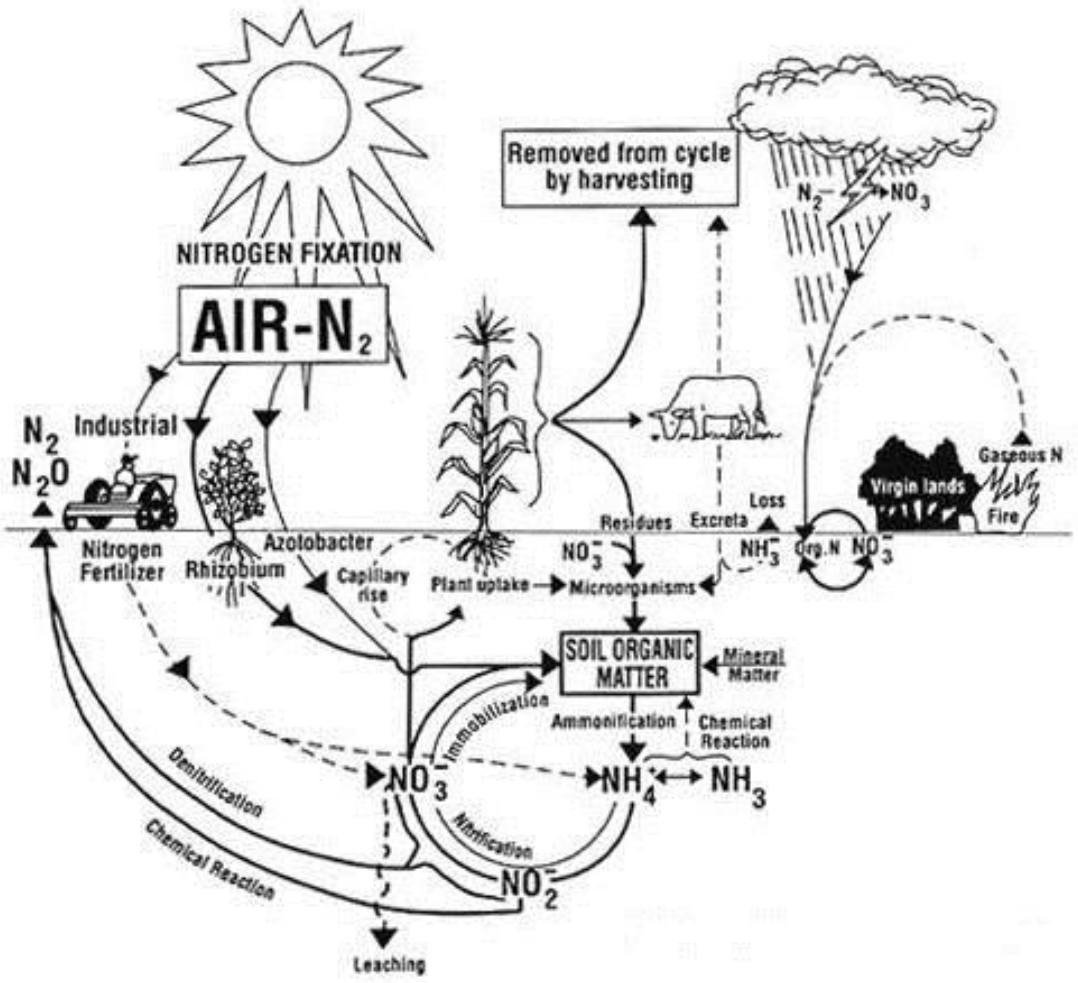

Figure 2.1 Summary of the nitrogen cycle (Brown et al., 1991)

As seen in Figure 2.1, the organic nitrogen gets into the sediment by animal excretion, decomposition of biomass, and agricultural activity, as well as through nitrogen fixation by plants (Brown et al., 1991). Organic and inorganic nitrogen are cycled by ammonification and immobilization; these are primarily bacterial processes (Burger et al., 2003). Processes such as denitrification and leaching cause nitrogen to be lost directly from the sediment, while processes such as the harvesting of crops remove nitrogen from a system at the soil surface. However, the main focus of this project was on processes within

the inorganic nitrogen pool. Inorganic nitrogen, most commonly found in the forms of ammonia, nitrate, nitrite, and nitrogen gas, are important building blocks in the life cycle of all animals. Nitrogen is necessary for the creation of amino acids in organisms, which mostly comprise proteins – as a result, bacterial biomass on average has a carbon to nitrogen ratio of roughly 5.4 (Nagata, 1986). Depending on the hydraulic state of the wetland, the majority of nitrogen in a system could enter through influent flow. Inputs for a body of water can include any streams feeding the body of water, runoff from surrounding land, and groundwater inputs. The majority of the nitrogen entering a system will be in the form of nitrate, since nitrate is the most mobile form of nitrogen (Webster et al., 2003). Meanwhile, ammonia is more labile, meaning that it is rapidly immobilized by various biological and physical processes (Webster et al., 2003). The last major source of nitrogen in a system, as mentioned previously, is decomposition of organic biomass. When organic matter decays, the decomposition and ammonification create ammonia, which is processed into nitrate and nitrogen gas by nitrification and denitrification, respectively. These processes are controlled by separate bacterial communities, namely nitrifiers and denitrifiers, as well as by physical processes. The bioavailability in soils is monitored by temperature, hydrologic fluctuations, water depth, electron acceptor availability, and microbial availability (Reddy et al., 2008). The bacteria-catalyzed nitrification and denitrification reactions are shown below.

Nitrification (Metcalf & Eddy, 2003)

$$NH_4^+ + 1.86O_2 + 0.098CO_2 \rightarrow 0.02C_5H_7NO_2 + 0.98NO_3^- + 0.094H_2O + 1.98H^+ \quad [1]$$

Denitrification (assuming acetate as the carbon source) (Metcalf & Eddy, 2003)

$$0.625CH_3COOH + NO_3^- \rightarrow 2N_2 + 1.25CO_2 + 0.75H_2O + OH^- \quad [2]$$

Nitrification, carried out by chemolitho-autotrophic bacteria, requires dissolved inorganic carbon or bicarbonate as a carbon source, as well as oxygen as an electron acceptor (Kowalchuk et al., 2001). Alkalinity is consumed throughout the entire process. Based on the pH dependency of alkaline species, the optimum pH for nitrification is 7.5 to 8.0 (Metcalf & Eddy, 2003). On the other hand, denitrification is a heterotrophic process which requires organic carbon and operates in anoxic conditions. Nitrogen exits a system through advective transport at the highest rate, but since advection is low in wetlands, biogeochemical processes tend to remove more nitrogen as retention time increases (Strand et al., 2013). Bacterial processes such as nitrification and denitrification could account for most of this biogeochemical nitrogen removal (Brix, 1993).

Diel Changes in Nitrogen Cycling

Inorganic nitrogen enters the nitrogen cycle when a net release of ammonia is observed when ammonification exceeds bacterial and algae nitrogen uptake – the ammonification process is at its peak during nighttime hours (Spears et al., 2008). Benthic invertebrates and heterotrophs under anaerobic conditions create most of the ammonia, yet bioturbation also contributes to ammonia flux, as various organisms disturb ammonia that had been collected in the soil (Frazier et al., 1996). During the daytime, nitrogen cycling is more affected by photosynthetic processes, yet the presence of sunlight does not alter the total nitrogen cycle (Spears et al., 2008). Based on previous experiments, release of ammonia from the sediment is regulated by the rate of organic debris reaching the sediment, decomposition rate, and the rate of ammonia being released to pore waters (McCaffrey et al., 1980). Pore water, or interstitial water, is located in the spaces between sediments. The nutrient concentrations of these areas often exceed those in surface waters (Frazier et al.,

1996). Due to the low advective transport, in wetland conditions nutrients are released from pore waters via diffusion and bioturbation. After sunrise, autotrophic production begins alongside the heterotrophic respiration that had dominated through the nighttime.

In order to decouple these processes, nitrification rates during both daytime and nighttime are compared—thus calculating net rates and gross rates. While nitrification rates slightly increase during the daytime, denitrification occurs at all hours (Spears et al., 2008). While each step of the nitrogen cycle is important to the health of wetlands, in the next section the most relevant components will be discussed.

Nitrification

Nitrification converts ammonia to nitrate as shown in Equation 1 (Metcalf & Eddy, 2003). The reaction is a two-step process accomplished by Ammonia-oxidizing Bacteria (AOB) and Nitrite-oxidizing Bacteria (NOB) through completion of the following chemical reactions (Metcalf & Eddy, 2003). In the first step of nitrification, ammonia is oxidized to nitrite (Metcalf & Eddy, 2003). AOB are ammonia-oxidizing bacteria that use the enzymes *amoA*, the α-subunit of ammonia monooxygenase, and hydroxylamine oxidoreductase (HAO) to catalyze these transformation reactions (Kowalchuk et al., 2001). The overall reaction for AOB is shown below, with inorganic carbon factored in for cell growth (Metcalf & Eddy, 2003).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad [3]$$

Several genes are responsible for the entire nitrification process, yet the gene of interest for nitrification measurement in this case is the *amoA* gene (ammonia oxygenase).

The ammonia oxygenase gene is the functional gene responsible for the first reaction of nitrification: conversion of ammonia to hydroxylamine (Kowalchuk et al., 2001). Figure 2.2 displays the enzyme interactions between the *amoA* and other sites involved with nitrification.

The *amoA* gene codes an important enzyme responsible for nitrification, but quantifying can have some issues. In general, two copies of *amoA* exist per nitrifier (Hommes et al., 1998). Therefore, if the number of *amoA* genes can be quantified in a sediment sample, the number can be indirectly related to the number of AOB present. Other gene fragments can be quantified, though the technology has existed for profiling bacteria based on 16S rRNA gene fragments of the desired enzyme for more than twenty years (Muyzer et al., 1993). For the purposes of this thesis, the *amoA* gene was chosen to be quantified. The reasoning for this decision was justified to ensure that this project could be most comparable with others.

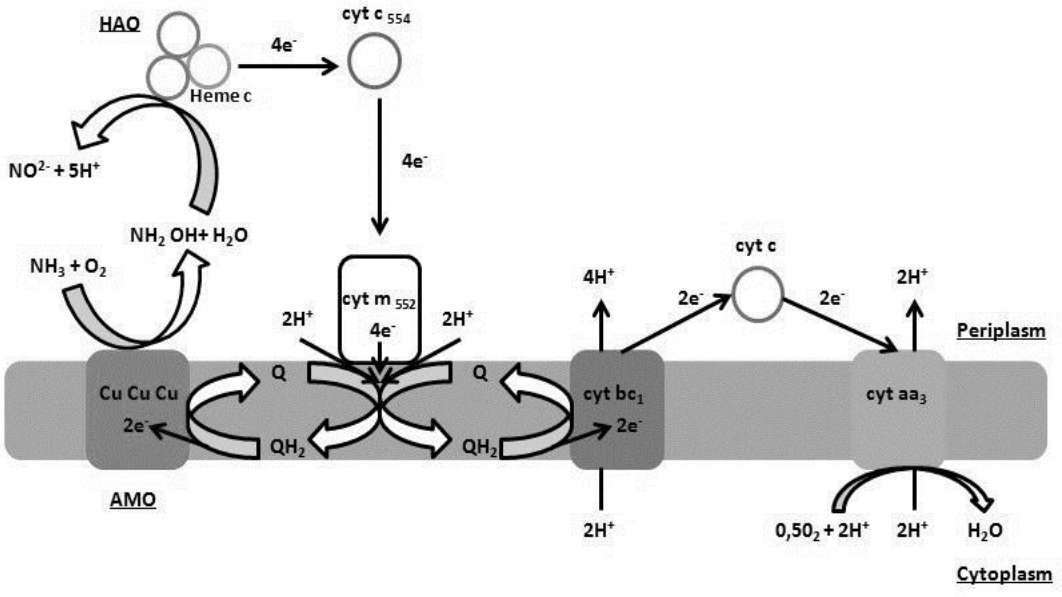

Figure 2.2 Coordination of nitrification enzymes (Byung et al., 2008)

Quantifying *amoA* as a count for AOB population has distinct advantages over using rRNA markers for quantification: a larger database for AOB genomes based off of the *amoA* gene exists than for 16S, *amoA* genes from all subunits of *amoA* can be amplified at the same time, and the close relation of *amoA* to the methane-oxidation gene *pmoA* allows for a description of the relative abundancies between the two populations (Kowalchuk et al., 2001).

In addition, profiling AOB populations via the *amoA* gene can be accomplished using sequencing techniques such as terminal restriction fragment length polymorphism (T-RFLP) (Osborn et al., 2000). T-RFLP involves digesting a target PCR product containing fluorescently labeled genes with restriction enzymes. The digestion is mixed with a DNA size standard and sent through capillary electrophoresis for laser detection of the fluorescent DNA fragments (Osborn et al., 2000). The identity of the bacteria present in the sample can then be determined based on the resulting electropherogram (Osborn et al., 2000). Using this technique, the following AOB species can be identified: *Nitrosomonas europaea/eutropha, Nitrosomonas oligotropha, Nitrosomonas cryotolerans, Nitrosomonas marina, Nitrosomonas communis,* and the genus *Nitrospira* (Siripong et al., 2007). These species of AOB, shown in Table 2.1, share the same functional class but may be present in wetland sediments based on the condition of the bacteria's surrounding environment (Koops et al., 1991). Included are the expected TRFLP peaks, as outlined in the methods section (Siripong et al., 2007). However, since TRFLP is an identification system based on the nucleotide size as opposed to nucleotide sequence, TRFLP can only be used as a general guideline for bacterial analysis. It is a streamlined method in which multiple samples can be analyzed over a short period of time. Ultimately, the results of TRFLP must be checked using DNA sequencing to confirm the identity of the targeted bacteria.

Table 2.1 Species of *Nitrosomonas* Determined by TRFLP

	Maximum ammonia tolerance*	**Salt requirement**	**Growth at 0°C**	**TRFLP Electropherogram peak**
N. eutropaea	High	No	No	219/270
N. oligotropha	Low	No	No	48/135, 354/135
N. cryotolerans	Mid	Yes	Yes	48/441, 354/48
N. marina	Mid	Yes	No	48/441, 48/135
N. communis	Mid	No	No	491/491

*High: > 400 mM ammonia, Mid: 100-400 mM ammonia, Low: <100 mM ammonia

(Koops et al., 1991)

The second step of the nitrification process involves oxidizing nitrite to nitrate as carried out by NOB, as shown by the following reaction (Reddy et al., 2008).

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad [4]$$

In this reaction, the oxygen is the electron acceptor – if oxygen is not present, it is inhibited. This reaction can be carried out both heterotrophically by the genus *Nitrobacter* or autotrophically by nitrite oxidizers such as *Nitrospina*, *Nitrococcus*, and *Nitrospira* (Burrell et al., 1998). The dominance of the NOB genus depends on the location—*Nitrobacter* has been found to be a ubiquitous bacteria, found in both sewage and marine environments, dry environments, and with a wide range of preferences for pH (Spieck et al., 2005). However,

Nitrospira tends to outnumber *Nitrobacter* when both communities are in competition (Spieck et al., 2005). *Nitrobacter* incorporate the enzyme nitrite oxidoreductase (NOR), while the other genera reduce nitrite using a nitrite-oxidizing system (NOS) (Spieck et al., 2005). The main focus of this study is on AOB because ammonia oxidization has been proven to be the rate-limiting step of nitrification (Wankel et al., 2011). However, AOB are not the only microorganisms capable of ammonia oxidation.

Archaea are a new domain of microorganisms that were identified as requiring their own classification in the late 1900s. Previously grouped together with bacteria because both domains are prokaryotic, these species were later found to incorporate eukaryotic metabolic processes, and thus need their own classification (Woese et al., 1990). Despite the classification difference, archaea share many of the ecological niches as bacteria, including nitrification. Ammonia-oxidizing archaea (AOA) have been found to outnumber AOB by a factor of 17 to > 1600 in semiarid soil samples, while a lesser ratio has been acknowledged for surface waters (Ergunder et al., 2009). In fact, studies of estuarine environments have shown AOB to be more prevalent than AOA (Wankel et al., 2011). Overall, AOA tend to proliferate in low ammonia concentration environments (Ergunder et al., 2009).

Many different organisms are capable of nitrification, and some studies have been made to compare the nitrification potential of these organisms. It was found that *Nitrospira*-type bacteria, as well as regions with more diverse AOA populations, tend to nitrify at a faster rate, normalized for population, than areas with more prevalent *Nitrosomonas*-style bacteria and less diverse AOA (Wankel et al., 2011). Of course, sediment conditions also play a part in the nitrification process.

Generally, nitrification occurs in the top ten centimeters of sediment as this region of the sediment has more exposure to oxygen than deeper layers and contains the highest

concentration of ammonia from decomposition (Kadlec et. al, 2009). Due to the high ammonia content of the top layer of the soil (from decomposition and ammonification of organic matter) and the higher surface area for bacteria to attach to, the nitrification rates of the sediment can be 3–4 times higher than the above water column (Wetzel, 2008). While oxygen presence and ammonia concentration dictate the rate of nitrification, several other factors come into play as well. For example, higher concentrations of dissolved oxygen can cause stimulated microbial growth, which can reduce ambient ammonia concentration, limiting the substrates for nitrification (Bernhardt, 2002). On the other hand, the types of sediment present can affect nitrification; soils with higher volatile solid concentrations contain more ammonia, which can more easily stimulate nitrification (Frazier et al., 1996). As long as the ammonia input to an area does not exceed the amount that can be nitrified by bacteria in that area, then the effects of excess ammonia should not occur. To quantify this maximum input level, various bacterial analyses must be completed on the sediment of an area.

Using genomic identification techniques for sediment bacteria such as gene quantification and identification could help determine how effective a target wetland could be for bioremediation (Zhu et al., 2001). These techniques could be applied to the FBWMA to help determine the wetland's capability for remediating ambient nutrients as the bacteria which facilitate nutrient removal are prevalent in all wetland sediments. In order to determine the number of *amoA* genes present, one must evaluate a DNA sample with real-time PCR (qPCR). qPCR measures fluorescent signals in conjunction with DNA replication to quantify the amount of a target gene in a sample (Dorak, 2007). The details of this process are covered in the methods chapter of this thesis.

Nitrification is so important and active in wetlands that municipalities have been

using wetlands as tertiary treatment systems for WWTP effluents (Brix, 1993). Early in the development of creating wetlands to remove effluent nutrient contaminants, nitrification was found to be the limiting step in nitrogen removal (Wittgren et al., 1995). Therefore, optimization of nitrification in wetlands should be a chief goal. More specifically, availability of electron acceptors, such as oxygen for nitrification, shows a high correlation to a wetland's capability for nutrient cycling (Kivaisi, 2001). As a result, studies have been made on how to more effectively deliver oxygen to the sediment to enable nitrification; one example involves examining the types of macrophytes which contribute the most oxygen to the root zones of the sediment (Allen et al., 2002). Better management of wetlands could eventually lead to stimulated nitrification and thus more efficient nitrogen removal.

A large body of literature, cited in the previous section, exists for the study of nitrification and the encouragement of this process for remediation in wetland conditions. Therefore, it is obviously important that nitrification should be considered as a primary driver of nitrogen disappearance, and this process should be considered as an assessment tool for wetland condition.

Denitrification

Competition occurs between denitrifiers and SAV, as the plants incorporate nitrate (as well as ammonia) from pore water in the sediment into the roots of the SAV (Brown et al., 1991). However, since denitrification can occur at all anoxic depths of sediment and nitrate uptake for SAV only occurs at the aerobic root zone, competition between SAV and denitrifiers is minimal (Allen et al., 2002). Anoxic zones of sediment include areas with less than 0.5 mg/L dissolved oxygen, yet still contain bioavailable nitrogen (Zogorski et al., 2006). Overall, the majority of the nitrate in the sediments is removed by denitrification

(Brown et al., 1991).

Nitrate in the sediment undergoes two separate processes—assimilatory nitrate reduction and dissimilatory nitrate reduction. Assimilatory nitrate reduction is facilitated by SAV and some microorganisms and requires reducing the nitrate into ammonia before incorporation into the biomass of the organism (DeBusk et al., 2001). On the other hand, dissimilatory nitrate reduction simply uses nitrate as an electron source through a couple reactions; dissimilatory nitrate reduction to ammonia (DNRA) reduces nitrate into ammonia, and denitrification reduces nitrate into nitrogen gas (DeBusk et al., 2001). All nitrate-reduction processes occur in anoxic conditions using a heterotrophic process. In sediments, denitrification is primarily carried out by heterotrophic bacteria. As a result, organic carbon is consumed in sediments by heterotrophic denitrifiers—linking the nitrogen cycle with the carbon cycle as highlighted by the overall denitrification reactions below (Metcalf & Eddy, 2003).

$$NO_3^- + 1.25CH_2O + H^+ \rightarrow 1.25CO_2 + 0.5N_2 + 1.75H_2O \quad [5]$$

Denitrification requires organic carbon to be used as an electron donor in order to complete reduction of nitrate to nitrogen gas. In fact, the necessary organic carbon can be consumed from a variety of sources, including dissolved organic carbon and methanol, as long as the carbon is bioavailable. In this way, carbon is linked to the nitrogen cycle in wetland areas. Carbon enters wetland areas through photosynthesis, dissolved organic carbon from inlet water, and from other various nonpoint sources, such as runoff (DeBusk et al., 2001). Accumulating organic matter then undergoes more carbon cycling through hydrolysis, where enzymes released from plants, fungi, and bacteria convert the high

molecular weight polymers into monomers and oligomers that can later be taken up by microorganisms (DeBusk et al., 2001). This step is considered the rate determining step of organic matter decomposition (DeBusk et al., 2001). The smaller organic molecules created by hydrolysis then undergo heterotrophic metabolism by the microorganisms present. This is where the nitrogen cycle comes in, because the rate of heterotrophic metabolism depends on the availability of electron acceptors; after oxygen, nitrate molecules are the most energetically favorable electron acceptors in anoxic environments (Wetzel, 2008).

However, partially due to the low flow rates of water in wetlands, the oxygen content for aerobic degradation is lower than in rivers or streams. Therefore, most of the organic matter degradation that occurs in wetlands is anaerobic, which occurs at a much slower rate than aerobic degradation. As a result, wetlands become a carbon sink—which can be advantageous for carbon dioxide removal from the atmosphere. However, when drained, wetlands become a source of carbon to the rest of the biosphere (DeBusk et al., 2001). Wetlands play a crucial part in the carbon cycle which can be tied to nitrification and denitrification processes.

Denitrification Biology

Denitrification is typically the dominant pathway for nitrogen removal from wetlands (DeBusk et al., 2001). This process is mediated by heterotrophic microorganisms in wetlands, although autotrophic denitrification coupled with sulfate reduction or metal reduction can also occur. So the rate of denitrification is regulated by the amount of carbon present (DeBusk et al., 2001). However, the rate of denitrification (0.003 – 1.02 g N m^{-2} d^{-1}) outstrips the rates of nitrification (0.01 – 0.161 g N m^{-2} d^{-1}) (DeBusk et al., 2001). The reason for such a higher rate of denitrification than nitrification could be the variety of

microorganisms which can facilitate denitrification. Organotrophs, chemolithotrophs, photolithotrophs, diazotrophs, archaea, and others are responsible for denitrification as each can share a common enzyme for denitrification (Kadlec et. al, 2009). Denitrification occurs as a multistep process, and the major four steps are shown below.

$$NO_3 \xrightarrow{\text{nar}} NO_2 \xrightarrow{nir} NO \xrightarrow{nor} N_2O \xrightarrow{nos} N_2 \quad [6]$$

Each step is catalyzed by a different enzyme: nitrate reductase (*nar*) converts nitrate to nitrite, nitrite reductase (*nir*) converts nitrite to nitric oxide, nitric oxide reductase (*nor*) allows the conversion to nitrous oxide, and last, nitrous oxide reductase (*nos*) completes the reaction by converting nitrous oxide into nitrogen gas (Bothe et al., 2007). The rate-limiting step for denitrification along this pathway varies based on the species of denitrifier (Carlson et al., 1983). Of the enzymes responsible for denitrification, the enzyme of interest for this analysis is nitrite reductase (*nir*), as the enzyme is easily quantified using qPCR.

Nitrite reductase exists in two different forms coded by the genes *nirK* and *nirS* (Bothe et al., 2007). To quantify denitrifying bacteria in sediment, the amounts of *nirK* and *nirS* genes were measured. Quantification of *nir* was chosen over 16S rRNA quantification because both the *nirS* and *nirK* genes appear to be polyphyletic, meaning that the two genes' phenotypes have converged despite not having common ancestors (Oakley et al., 2007). Therefore, using 16S rRNA to measure nitrite reductase would measure the enzymes nonspecifically (Oakley et al., 2007). These *nir* genes fulfill the same role, but usually one is far more prevalent than the other in ecosystems; *nirS* is more common in marine and estuary environments while *nirK* genes dominate terrestrial environments (Jones et al., 2010). Salinity may be a factor in this difference in biome presence as *nirS* genes tend to be more abundant

with higher salinity (Jones et al., 2010). In addition, it was found that some species of AOB contain *nirK* genes. This could result in higher amounts of *nirK* in areas with greater amounts of ammonia flux (Oakley et al., 2007). Each gene represents a different enzyme responsible for nitrite reductase—*nirK* indicates the enzyme which reduces nitrite using a copper subunit, while *nirS* corresponds to an enzyme which reduces nitrite using a cytochrome cd_1 catalyst (Bothe et al., 2007). Despite the structural differences, these enzymes remain functionally equivalent. Denitrifying bacteria usually contain either the *nirK* or *nirS* enzyme; only one copy of one or the other can be found in each denitrifying bacterial genome (Bothe et al., 2007). Therefore, a good approximation of the amount of denitrifying bacteria can be directly correlated to the number of *nirK* genes present in addition to *nirS* genes. The amount of denitrifying bacteria can later be correlated to serum bottle test results to get a measurement for denitrification based on the amount of relevant bacteria present.

Phosphorus Cycling

Phosphorus Sources in Sediments

Phosphorus exists in wetlands as organic phosphorus or inorganic phosphorus. Organic phosphorus can be present in a variety of different molecules, while inorganic phosphorus is mostly composed of phosphate (PO_4^{-3}). Inorganic phosphorus exists as a solution in the water, attached to iron and aluminum in sediments, or attached to calcium and magnesium in sediments (Reddy et al., 1999). Unlike nitrogen or carbon, phosphorus accumulates in wetlands due to not having a means of gaseous release other than through phosphine emission (Han et al., 1988). However, phosphine (PH_3) emissions are mostly in the nanogram and pictogram per m^2 per day range, making it negligible compared to the amounts of phosphorus measured in this study (Han et al., 1988). While this limits the

exodus of phosphorus from a system, the benefit of low phosphine emissions is that this chemical is a toxic gas that is deadly in low doses (Han et al., 1988). Instead, phosphorus cycles between the water column and sediment before finally settling into the sediment through both biotic and abiotic processes. Meanwhile, inputs from WWTP effluents and runoff continually add phosphorus to this cycle (Reddy et al., 1999). For this reason, limiting the amount of phosphorus being released from municipal and agricultural sources is the most direct way of avoiding phosphorus accumulation in the environment. Figure 2.3 gives a clearer outline of the phosphorus cycle in water.

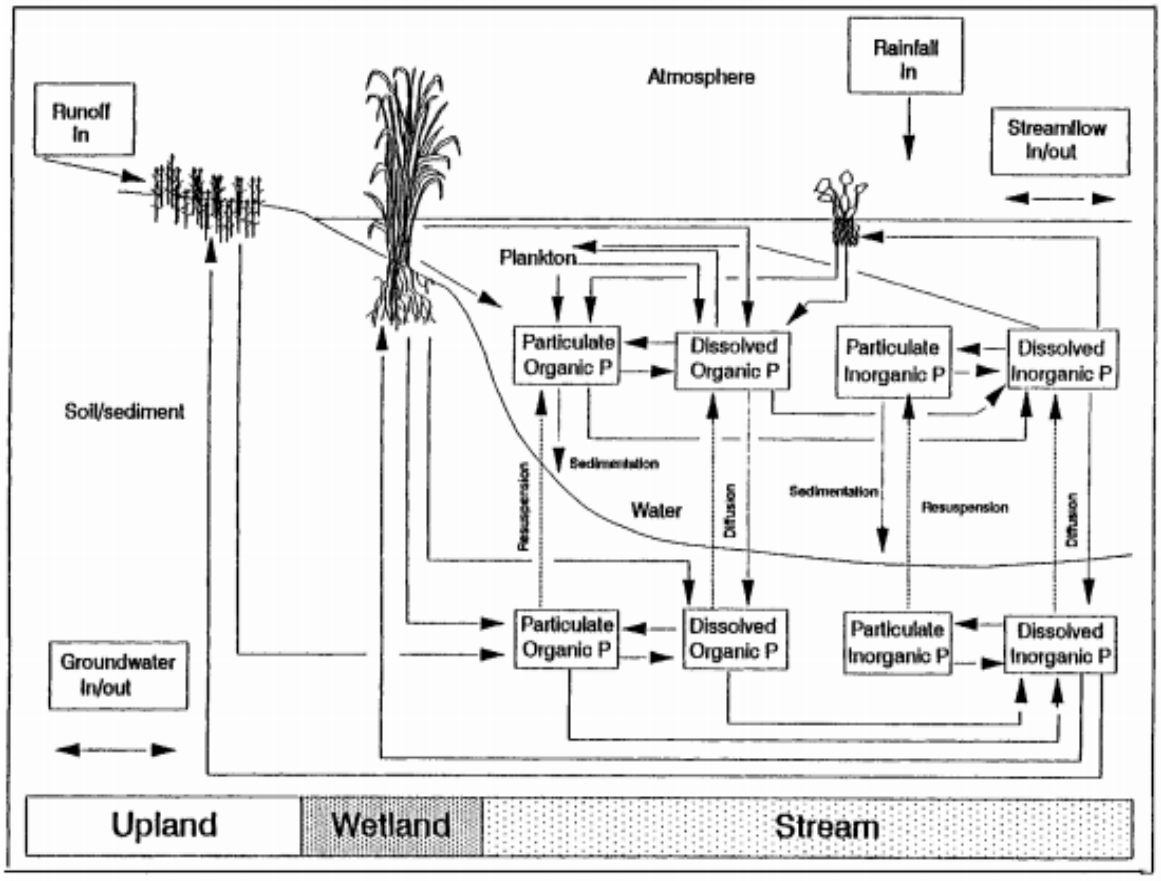

Figure 2.3 The Phosphorus cycle in water systems (Reddy et al., 1999)

In order to understand the cycle, one must be able to differentiate between particulate and dissolved phosphorus, as well as inorganic and organic phosphorus. Just below the sediment surface phosphate exists as dissolved in interstitial waters, while deeper in the sediment phosphorus exists primarily in particulate form (Reddy et al., 1999). Particulate forms of phosphorus include phosphorus in organisms, mineral phases of rock and sediment on which phosphorus has formed complexes, or phosphorus absorbed into dead organic matter (Wetzel, 2008). These forms constantly cycle between being dissolved and particulate, moving through biotic and abiotic pathways such as desorption, dissolution, ligand exchange, or enzymatic hydrolysis (Reddy et al., 1999; Wetzel, 2008). Inorganic phosphorus is the bioavailable form of phosphorus, commonly known as phosphate, and organic phosphorus requires transformation to phosphate in order to become bioavailable (Reddy et al., 1999)

Particulate phosphorus enters the sediment using five major processes:

1. Sedimentation of phosphorus attached to turbidity in water.
2. Adsorption or precipitation of phosphorus with inorganic compounds.
3. Sedimentation of phosphorus with autochthonous organic matter.
4. Sedimentation of phosphorus with allochthonous organic matter.
5. Phosphorus consumption through algal photosynthetic processes, followed by death and decomposition of algal organisms (Wetzel, 2008).

Allochthonous organic matter is organic matter transferred from terrestrial sources into water, while autochthonous organic matter is created through phytoplankton production (Wetzel, 2008). As a result, most of the phosphorus in the water column, as well as some of the phosphorus in the pore water, remains in a dissolved form (Reddy et al., 1999). Inorganic phosphorus in the pore water can then chemically react with functional

groups of different compounds in the sediments to become less mobile until physical conditions such as pH and dissolved oxygen concentration change (Reddy et al., 1999).

Phosphorus Forms and Fate in Wetlands

Due to the different forms of phosphorus present in sediments, more factors control the fate of phosphorus in the sediment than in the water column. To help understand which mechanisms control the exchange of particulate phosphorus for dissolved phosphorus, phosphorus speciation of sediment can be undertaken.

Phosphorus speciation involves identifying the species of particulate phosphorus in sediment through a sequence of reactions designed to solubilize phosphorus based on pH. Phosphorus can be described as loosely sorbed—meaning soluble and/or bioavailable for metabolic processes. In addition, phosphorus can be attached to aluminum and iron in acidic conditions as well as bound to calcium and clay in alkaline conditions (Reddy et al., 1999). The remainder of phosphorus is described as residual: difficult to make soluble through pH changes. As photosynthetic processes change the pH of the water column and sediment changes throughout the day, phosphorus attached to calcium/clay and Fe/Al can be released into the pore water or water column. However, the largest contributor of phosphorus release from sediment is particulate phosphate attached to iron and aluminum (Wetzel, 2008). Iron and aluminum complexes with phosphate are reduced in anoxic conditions while calcareous soil maintains phosphorus in anoxic conditions (Reddy et al., 1999). As a result, sediment can display absorption characteristics similar to the Langmuir isotherm based on the amount of iron and aluminum present (Reddy et al., 1999)

After particulate phosphorus is solubilized, it can be released into the water column through a number of processes. Bioturbation, or disturbance of sediment by animal or insect

activity, causes the largest amount of phosphorus resuspension into sediment (Wetzel, 2008). Diffusion, wind-induced turbulence, gas ebullition, cyanobacterial processes, and rooted aquatic plants also play their own role in allowing phosphorus to enter the water column (Wetzel, 2008). In fact, rooted plants play a very large role in the wetlands phosphorus cycle. Emergent plants absorb all the phosphorus for growth needs from bioavailable forms of phosphorus in the sediment (Wang et al., 2008). This phosphorus exchange takes place at the root zone, which works well because the roots create an oxygenated zone in the sediment, thus releasing phosphorus from iron and aluminum compounds (Wang et al., 2008). After death and decay, however, most of the phosphorus returns, and remains, in the sediment until released (Wang et al., 2008). Overall, studies have shown that the most important variable in determining sediment release capabilities of wetlands is the dissolved oxygen concentration of the overlaying water (Wang et al., 2008). However, the dissolved oxygen concentration which allows for the most phosphorus released depends on site-specific information such as phosphorus speciation and the makeup of biological communities in the wetland (Wang et al., 2008). All in all, the phosphorus cycle is loosely associated with the carbon and nitrogen cycles in the wetlands, but is also a necessary process to understand in order to prevent eutrophication.

Conclusions from Literature Review

Wetland nutrient dynamics are complicated processes composed of many interconnected processes involving water chemistry, biology, and physical conditions. The concept of using a multimetric index to help simplify which factors are important in explaining wetland health is a paramount idea as long as the qualifications of a healthy wetland are well understood. Included in this understanding of wetland health should be a

characterization of optimal sediment performance. Sediment which can maintain proper nitrogen- and phosphorus-cycling function during elevated nutrient loads would be the most beneficial in the Farmington Bay Wetlands as Salt Lake City and the surrounding areas continue to grow in population. While the phosphorus cycle displays many sophisticated physical chemistry considerations, the complexity of the nitrogen cycle with respect to bacterial contributions makes this nutrient the chief focus for this study. Additionally, nitrogen has been found in previous studies to be the limiting growth factor for the problematic algal blooms in Farmington Bay, making understanding nitrogen in this wetland the main objective (Wurtsbaugh et al., 2004). Hopefully, enough information will be found by this study to justify incorporating the sediment contributions to nitrogen- and phosphorus-cycling into a new, improved MMI.

CHAPTER 3

MATERIALS AND METHODS

Site Details and Flow Regime

Sites for this study were chosen from a list of sites previously sampled by UDWQ. The ambient nutrient concentrations were provided by UDWQ to help select a list of sites with a desired gradient. The sites with high nutrient concentrations in the Farmington Bay Wildlife Management Area selected for this project included FBS and Unit 2, the mid-range concentration sites were Unit 1, Turpin, and FB SE Unit 1, and the low concentration sites were FB NE Pond and South Area. These concentrations were taken from former UDWQ sampling events and a 2013 University of Utah sampling event which led to the development of the sampling standard operating procedure. Ambassador Duck Club Pond #1 and Bear River Nature Preserve Unit 5C both had mid- to low-range nutrient concentrations and were included to compare against FBWMA sites. Table 3.1 depicts the coordinates of sampled sites and Figure 3.1 locates sites on the map.

The ambient nutrient concentrations are partially controlled by influent streams into each site. Not shown in Figure 3.2, Ambassador Duck Club receives water from Surplus Canal, which branches off from the Jordan River near 2100S and I-15 in Salt Lake City, while the Farmington Bay Wetland Management Area (FBWMA) sites receive influents from a variety of sources. The variety of water sources and water quality into FBWMA make it a diverse wetland laboratory.

Table 3.1 GPS Coordinates of Sampling Sites

Site	**Latitude**	**Longitude**	**Type (*Alternative Futures Analysis, 2010*)**
NE Pond	40°57'14.41"N	111°54'47.17"W	Emergent/Impounded
Unit 1	40°56'37.49"N	111°55'43.35"W	Impounded
Turpin	40°54'36.38"N	111°58'49.95"W	Impounded
Unit 2	40°55'30.12"N	111°56'21.00"W	Impounded
South Area	40°54'31.90"N	111°56'22.63"W	Emergent/Impounded
SE Unit 1	40°55'6.80"N	111°55'12.50"W	Emergent/Impounded
FBS	40°54'0.10"N	111°56'25.10"W	Emergent
Bear River Unit 5C	41°25'25.34"N	112°5'37.58"W	Impounded
Ambassador Duck Club Pond #1	40°50'52.72"N	112°1'42.55"W	Emergent

Figure 3.1 Farmington Bay Wetlands site locations

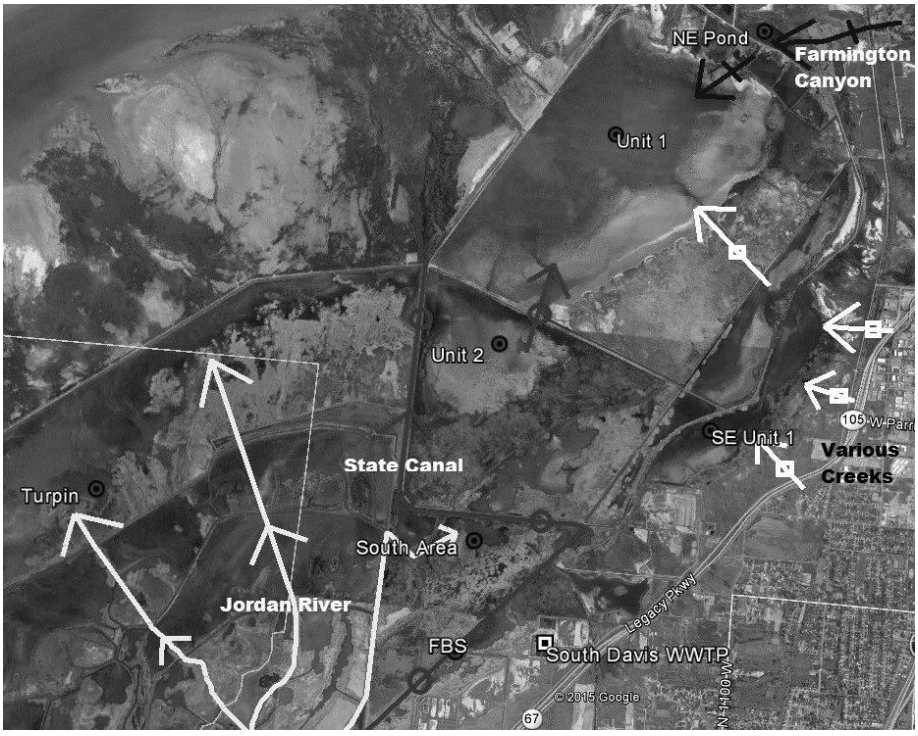

Figure 3.2 Farmington Bay Wetlands approximate flow regime

Before the Jordan River enters the FBWMA, State Canal branches off just west of Legacy Parkway to feed the southeast side of Farmington Bay. The first site encountered by State Canal is FBS, where the effluent from the South Davis WWTP discharges into the canal; this and other upstream conditions create high nutrient concentrations in State Canal, and therefore the subsequent sites. The canal ventures north, then west, where it feeds Unit 2, causing high nutrient concentrations. After Unit 2, State Canal discharges directly into the Farmington Bay. The third site with the highest nutrient concentrations is SE Unit 1. This site is actually fed by multiple drainage canals and creeks from Bountiful and the Wasatch Front. In addition, SE Unit 1's close proximity to a landfill contributes to the high nutrient concentrations. As for the remainder of the Jordan River water, it feeds into wetlands managed by Newstate Duck Club until eventually feeding water into Turpin and South Area. These sites display lower nutrient concentrations than the State Canal sites and SE Unit 1 because of the greater amount of time the water spends in prior wetlands before reaching Turpin and South Area. On the other hand, Unit 1 and NE Pond receive water from the northern side of FBWMA. NE Pond receives water directly from Emigration canyon, while Unit 1 receives water from a variety of different sources, including NE Pond effluent. As a result, both of these sites display low nutrient concentrations. Lastly, Bear River Nature Preserve Unit 5C receives water from the Bear River itself. In Figure 3.2, the yellow arrows and lines represent the Jordan River influent, red arrow indicates State canal influents, the white arrows indicate multiple creek influents, and the blue arrow indicates any influents from Farmington Canyon. Farmington Canyon provides water that is of the best quality, as it encounters a lower population density along the way to the wetlands. Multiple municipal effluents empty into both the Surplus Canal and the Jordan River. The water quality often reflects the source, as discussed later.

Task 1: Design and Fabricate In Situ Flux Chambers and Flux Measurements

Flux Chamber Creation and Operation

The flux chambers were constructed from transparent acrylic sheets and were 10”×10”×36” in dimensions. After construction, chambers were tested in the lab for water tightness. A practice sampling event was also conducted before the commencement of actual experiments for training purposes and to ensure all quality controls. At each site, two sediment chambers and two water column (WC) chambers were deployed to measure the daytime nutrient dynamics at the sediment-water interface and within the water column. *Furthermore, flux experiments were conducted under ambient conditions as well as under nutrient spiked conditions* (detailed later in this section). The sediment chamber had both an open top and open bottom to measure nutrient dynamics in the water column while interacting with sediments. The WC had an open top and closed bottom to measure nutrient dynamics in the water column only. The transparent open-top chamber design allowed inclusion of the entire water column accounted for sunlight exposure (i.e., photosynthesis), allowed gases to escape the chamber, and permitted easy access to the chamber for mixing and sample collection.

The sediment chambers were installed before the WC chambers. If the sediments were noticeably disturbed during chamber installation, the chambers were moved to a new location. A small amount of sediment disturbance at the chamber walls was unavoidable since the chamber was used to physically isolate both the sediments and water column. If the sediments within the chambers became resuspended, this skewed results due to the release of sediment pore water and artificial turbidity.

The sediment chamber was pushed into the sediment 10-15cm in to isolate the water column above the contained sediments (see left side of Figure 3.3).

Figure 3.3 Installing sediment chamber (left),
both chambers deployed (right)

The thin acrylic easily penetrated the fine sediments with minimal disturbances. Most GSL wetlands have a clay layer underlying the surface sediments that provided an excellent foundation to support the chambers during light winds.

The WC chamber had a closed bottom and open top to isolate the water column only. The control chamber was gently filled with ambient water to the same level as the sediment chamber (i.e., height of ambient water column). This was accomplished by carefully filling the chamber sideways while collecting water beneath the surface, or by filling the chamber using the submersible pump placed alongside the canoe while positioning the pump at variable depths during chamber filling. The pump method is recommended if there is not enough room to fill the WC chamber without disturbing sediments. If the sediments became disturbed while filling a WC chamber, the pump was moved to the other side of the canoe to allow filling of the chamber with 'clean' water. The working volumes of the

sediment and WC chambers mimicked ambient conditions by having a water height in the chambers equal to the depth of the water column. Figure 3.3 (right) shows a WC and sediment chamber deployed next to each other.

Each chamber had its own submersible pump, with the WC pumps being placed directly on the bottom of the closed chamber. The sediment chambers required adjusting a 'hanger' on the PVC tube, and adjustment was based on how far the sediment chamber was pushed down into the sediments. Ideally this height should not change, but in very mucky sediments, the chamber may be inserted 20–30cm deep into the sediments. This would result in the pump being placed directly into the sediments when hanging if not repositioned, requiring the chamber to be installed at another location due to sediment disturbances. Before placing the pump in the sediment chambers, the depth of the submersible pump was tested by hanging the pump from the outside of the chamber while confirming that the bottom of the pump was roughly 1" above the sediments. If the pump was found to enter the sediments outside of the chamber, the hanger was shortened in length and 'dip cleaned' before installation in the chamber. The pump outlet tubing had a ball valve installed near the top of the PVC tube. The valve was closed at least halfway to ensure the circulating water did not disturb the sediments when the pumps were initially turned on. After all chambers and pumps were installed, a visual observation was made on indicators such as high turbidity, green water, surface foaming, floating periphyton mats, the presence of carp, etc. These variables may become valuable when analyzing data in the future.

This study incorporated nutrient spiking to observe nutrient concentrations previously below detection limits and to witness any change in nutrient uptake kinetics due to increased nutrient loads. The first four hours of the study were conducted under ambient conditions, while the last four hours involved spiking the chambers to a calculated

concentration of 0.5 mg/L NH_3-N, 0.5 mg/L NO_3-N, and 0.1 mg/L PO_4-P. Four samples were taken during both ambient and spiked conditions. Samples were collected at consistent time intervals, but collecting a sample slightly earlier or later than planned would not influence the final calculations. Longer chamber deployment times are preferred, if feasible, to capture sediment and WC nutrient dynamics in ponds having very low ambient nutrient concentrations. As a general rule, higher ambient nutrient concentrations required less time to observe a change in dissolved nutrients.

Before taking water samples, the chambers were gently mixed via submersible pump to account for potential stratification in the quiescent chambers. The flow of water was adjusted via the ball valve and needed to be throttled down when sampling in shallow ponds, or increased if the water was relatively deep. The pumps were all powered at the same time by manually connecting the positive and negative wires to a deep cycle 12V battery. Pumps were powered for 10 minutes to ensure complete and consistent mixing in the chambers before each sample. This means that constant mixing was not employed while the chambers were sitting in wetland sediments. The idea was not to continually mix the chambers, such as the approach when sampling rivers, but to ensure well-mixed conditions within the chamber prior to sampling. Care was taken not to allow pumps to resuspend any sediments. If turbid conditions were observed in the clear pump tubing, the valve was immediately throttled down. If the sediments were accidently suspended, sampling was aborted immediately and chambers were deployed at a new location at the same site. Furthermore, stakes were used (as shown in Figure 3.4) to make sure chambers did not tilt or move during sampling. The stakes were installed by hammering into the ground in a controlled manner as to not disturb sediment near the sampling chambers. Stakes were also installed to prevent any collisions of the sampling canoe with the sampling vessels.

Figure 3.4 Stake bulwark protecting 8 chambers

Samples were directly collected using the circulation pump tube. The outlet of the tubing above the water was carefully lifted to fill a water quality (WQ) sampling container. If water depths were shallow, the valve during sampling was throttled down to avoid the stream of water disturbing the sediments due to the additional head of the recirculating water associated with collecting a WQ sample near the top of the chamber. Following WQ sample collection, the container was immediately capped and stored on ice in a Coleman cooler.

Nitrate, nitrite, and phosphate concentrations were analyzed with a Methrohm 883 plus Ion Chromatograph using EPA Method 300.0 for Determination of Inorganic Anions

by Ion Chromatography. Ammonia concentrations were measured using a HACH TNT 830 ULR Ammonia Kit. All water samples were analyzed within 24 hours of collection.

DO/pH/Temperature Measurement

Each time a sample was collected, the dissolved oxygen, pH, and temperature of the water column were measured with a HACH HQ 40d DO/pH/Temp. A three-point pH calibration of the probe was conducted before arrival to the field for each site, and proper operation and maintenance consideration taken. Results were recorded for each sampling time along with nutrient concentrations to statistically distinguish trends. Changes in dissolved oxygen, oxygen saturation, temperature, and pH were calculated as a difference between the final sampling time and the initial sampling time for later statistical analysis.

Nutrient Flux Calculation

The WC rates and sediment fluxes are reported based on concentrations greater than analytical detection limits. The rate of change of dissolved nutrients for each chamber is calculated using the slope of the concentration (mg/l) versus time (day). All raw data and regressions are reported in the units of mg/L/day, and the final rates and fluxes in terms of $g/m^3/day$ and $g/m^2/day$. The WC rate was initially calculated since the field observed rate describes the nutrient dynamics occurring in the water column.

$$WC_{light} = \frac{dC}{dt} \quad [7]$$

WC_{light} = WC nutrient rate during daytime conditions ($g/m^3/d$)

dC = Change of nutrient concentration in chamber (mg/L)

dt = length of sampling event (day)

Before the flux was calculated, a linear regression of the nutrient concentration over time was taken. When plotting time versus nutrient concentration, a linear relationship must be statistically significant before concluding that nutrient changes are actually taking place in the WC or sediment column. For this study, if the R^2 of this regression was > 0.7, it was considered a significant enough trend to continue with sediment flux calculation. The value of > 0.7 for an acceptable R^2 range was selected from a range of 0.6-0.79 that is typically denoted as having "strong" correlation in a variety of fields (Evans, 1999). A midpoint of that range was chosen to account for the complexity of wetland biogeochemistry while still maintaining an obvious linear regression.

The sediment nutrient flux was calculated next by subtracting out the activity in the WC and normalizing the chamber working volume to the area of sediments enclosed in the chamber. Since the entire depth of the WC is used, the normalization factor becomes equal to the depth of the WC in meters. Note that dC/dt and WC_{light} are in the units mg/L/day and $g/m^3/day$, which are equivalent.

$$Sed_{light} = \left(\frac{dC}{dt} - WC_{light}\right) \times \frac{V}{A} \quad [8]$$

Sed_{light} = Sediment nutrient flux during daytime conditions ($g/m^2/d$)

V= Volume of water within chamber, varies with depth (L)

A= Sediment surface area within the chamber (0.0645 m^2)

$$d = \frac{V}{A} \times \frac{m^3}{1000\ L} \quad [9]$$

d = depth of ambient WC (m)

A negative rate or flux occurs when nutrients are being removed from the ambient water, and a positive rate or flux occurs when nutrients are being added to ambient water. After calculation of WC_{light} and Sed_{light}, the two parameters can be compared directly by normalizing one of the parameters to water depth. A WC aerial flux can be expressed by multiplying WC_{light} by the water depth. The Sed_{light} can be expressed as a rate influencing the ambient water by dividing by water depth. Using these relationships, the data collected were used to perform wetland pond mass balances, identify whether the sediments or WC were responsible for the majority of nutrient cycling, and to compare nutrient dynamics with other GSL wetlands and literature. To calculate the minimum detection limits for the nutrient flux studies, the same calculations were carried out with concentration changes just outside the standard deviation for IC and spectrophotometric determination. For example, since the standard deviation for nitrate was 0.03 mg/L NO_3-N, this change for each sampling time of nitrate at a site was used to calculate change in concentration over time and inserted into the above equation.

Nonpurgeable Dissolved Organic Carbon (npDOC) in the Water Column

Within 24 hours of sampling, npDOC was analyzed for every sampling time with the Shimandzu TOC-V total organic carbon analyzer while also incorporating a Shimandzu ASI-V auto-sampler. This way, up to sixty samples at a time could be loaded and analyzed automatically. To remove inorganic carbon, HCl was added to each water sample to maintain pH 2.5 +/- 0.1. All input water streams to the TOC-V were kept at pH 2.5 as well, per instructions from the manufacturer. An organic carbon calibration curve was created for every operation of the machine; the concentrations used were 1 mg/L C, 5 mg/L C, and 10 mg/L C. The same policy of including blanks before and after a sample set and between

every ten samples. Check standards of 10 mg/L C were included for every ten samples, as well. The same quality control procedures were carried out as with TOC of sediment.

Task 2: Conduct P Speciation Using a Sequential Extraction Technique and Determine Other Parameters in Sediment Core Samples

Sediment Core Collection

Along with water quality sampling, sediment samples were taken from each site to be used for future analyses and DNA extraction. Sediment core samples were taken using a modified KB coring device, as shown in Figure 3.5.

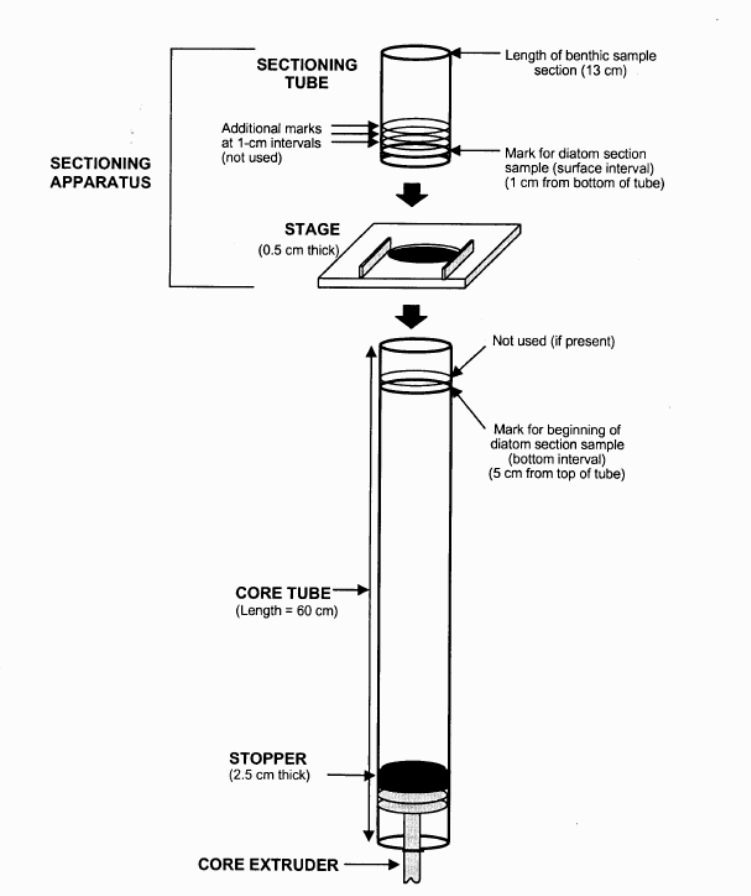

Figure 3.5 Modified KB sediment core device

The top 0–5 cm of sediment was collected and stored on ice in 200 mL plastic bottles to be used for analysis within 24 hours. Sampling involved inserting the corer into the sediment to a depth of around 20 cm. Water was filled to the exposed top of the corer until the water level reached the top. A rubber stopper was put into the top of the tube to ensure that water tension would keep the sediment in place as it was removed from the surrounding sediment. A plunger was then inserted into the sediment-filled end and pushed so the water exited the tube and left the top 0–5 cm of sediment within reach to be collected. The top 0–5 cm of sediment was collected with a spoon and placed in the plastic sample bottle for transport to the lab for analysis.

TS/VS

Standard Methods for the Examination of Water and Wastewater was consulted as the procedures for total solids (TS) and volatile solids (VS) were taken from section 2540 (American Public Health Association, 2012, p. 51). Total solids were measured as the percentage of mass which remained in the sample after evaporating water at 105°C overnight, while volatile solids were measured as the amount of solid organic compounds which were removed after ignition at 550°C. Special consideration was taken at each step to make sure all water had evaporated and organic solids burned away.

Total Organic Carbon in Sediments

The total organic carbon content of the sediment was measured using the Shimadzu SSM-5000A solid sampling module with a TOC-V total organic carbon analyzer. Proper operation and maintenance techniques were carried out throughout operation of the analyzer in order to determine only the organic carbon content of the sediment without inclusion of

any inorganic carbon. In order to achieve this, the sediment was first dried at 105°C overnight and pulverized before analysis. Sample preparation also included lowering the pH to below 2.5 using HCl on a heating block—this way all inorganic carbon in the sediment exited as carbon dioxide. Samples with high clay content required extra acid to remove all alkalinity.

Sample weight was kept at 200 mg and duplicate samples were taken for each site. A calibration curve was created at the start of each TOC run—this included making samples of 1 mg, 5 mg, and 10 mg of carbon, using acetate as a standard. The calibration curve was used as a quality control procedure to ensure the carbon being measured was accurate. Two sample blanks (0 mg carbon) were analyzed as both the first two and the last two samples; one blank was measured between every ten samples. Last, a 10 mg carbon from acetate check standard was observed for every ten samples as well. Check standards were expected to be within 15% error of the expected 10 mg C concentration, and deviation from this caused the experiment to be repeated and/or source of error looked into.

Phosphorus Bound to Minerals in the Sediment

For this analysis, total phosphorus was measured alongside phosphorus speciation experiments using different procedures. The sum of the phosphorus species should fall within 20% error when compared to a site's total phosphorus amount for the experiment to be deemed a success. The high percent error rate is due to the difficulty and complexity of both procedures.

For both total phosphorus and phosphorus speciation, a known mass of wet sediment was dried to remove moisture. For phosphorus speciation, it should be noted that phosphorus can be attached to different molecules in sediments. Phosphorus speciation was

tested sequentially, starting with loosely sorbed P, clay and Al/Fe-bound P, Ca-bound P, and residual P. Loosely bound phosphorus, which is the most bioavailable and accessible of the phosphorus species in sediment, can be released from sediment by simply exposing the sediment to sodium chloride under anoxic conditions. Clay and Al/Fe-bound phosphorus can be removed by exposing the sediment to basic conditions and shaking overnight. On the other hand, Ca-bound phosphorus requires a lower pH for release. Last, residual phosphorus includes phosphorus species which are less easily freed—this species requires drying the sediment at 105°C, followed by igniting at 550°C and boiling in HCl. After releasing the phosphorus into the step's supernatant, the concentration of the phosphorus was measured using HACH PhosVer 3 Phosphate Reagent Kit with a spectrophotometer at 880 nm wavelength. Each step required a different amount of dilution to be within reaction concentration for the PhosVer 3 kits.

Before running the experiment, a calibration curve was created for the PhosVer 3 kit packets; absorbance was measured against phosphorus concentration (in the form of potassium phosphate) to create the following equation.

$$[P] = Abs\ x\ slope \quad [11]$$

$$[P] = Concentration\ of\ PO_4 - P\ in\ sample\ \left(\frac{mg}{L}\right) Abs$$

$$= Absorbance\ measurement\ from\ spectrophotometer$$

$$= slope\ of\ absorbance\ versus\ concentration\ calibration\ curve$$

This step must be carried out whenever using a new spectrophotometric reagent. Interestingly, the slope found for the PhosVer 3 kits was 1 ppm/ABS. For each phosphorus speciation step, the following equation was used to convert the measured absorbance to a

measure of mg P/kg dry sediment.

$$m_P = \frac{V * Dilution\ Factor * [P]}{m_{sed}} \quad [12]$$

$$m_P = Mass\ of\ phophorus\ from\ speciation\ step$$

$$V = Volume\ of\ supernatant\ (L)$$

$$Dilution\ Factor = Amount\ of\ dilution\ required\ for\ speciation\ step$$

$$[P] = PO_4 - P\ Concentration\ found\ from\ absorbance\ equation\ \left(\frac{mg}{L}\right)$$

$$m_{sed} = Initial\ mass\ of\ sediment\ (kg)$$

The mass of phosphorus per dry sediment could then be summated and compared to the total phosphorus analysis as a quality control check.

Total Phosphorus

For total phosphorus, the soil was digested with perchloric and nitric acid, pH adjusted, and the phosphorus measured with a spectrophotometer through the ascorbic acid method. The digestion step is borrowed from *Standard Methods for the Examination of Water and Wastewater* and closely follows the "Perchloric Acid Digestion" method for total phosphorus measurement in section 4500-P (American Public Health Association, 2012). After several digestion step iterations, this digestion was chosen because it accounts for the highest amount of recovery as compared to phosphorus speciation.

The digestion step involves boiling a known weight of dry sediment in perchloric and nitric acid—each sample was run in duplicate. This step is followed by a heavy neutralization step using NaOH as the ascorbic acid reagent step, for determination of phosphorus by spectrophotometry requires neutral pH. The samples were then diluted and

brought back to 50 mL for the ascorbic acid mixed reagent step. The mixed reagent can be made using the following chemicals.

- 100 mL 5N Sulfuric Acid
- 60 mL Ascorbic Acid – prepared by mixing 8.8 g in 500 mL deionized water
- 10 mL Antimony Tartate – prepared by mixing 1.3715 g in 500 mL deionized water
- 30 mL Ammonium Molybdate – prepared by mixing 20 g in 500 mL deionized water

After adding 8 mL of mixed reagent to the 50 mL sample, the reaction was carried out for twenty minutes. Afterwards, the absorbance of each sample was measured at 880 nm and recorded.

The same calibration curve creation step as for phosphorus speciation is recommended for total phosphorus. In fact, the same equations can be used as before. It should be expected that the total phosphorus amount in the sediment should be within +/- 20% of the overall total phosphorus found from the speciation step.

Task 3: Determine the Rates of Nitrification and Denitrification Using Serum Bottle Tests

The use of serum bottle tests in this project had a three-fold purpose: to measure nitrification and denitrification rates in sediments, to estimate a kind of nitrogen balance of sediments for each site, and to indirectly serve as a way to measure pore water nutrient concentrations. However, one of the most important and basic steps in each of the analyses included determining the total and volatile solid composition of the sediment.

Pore Water Concentrations

Pore water concentrations were calculated using the following equations; the nutrient concentrations were taken from unspiked serum bottle test samples, as will be described later. In this case, 50 mL deionized water (pH ~ 7) was added to 10 grams of sediment, made into a slurry, and a 10 mL sample was immediately filtered for analysis by IC in order to prevent major concentration changes due to desorption. The following equations were used to indirectly measure pore water nutrient concentration from serum bottle test results.

$$V_{Pore\ Water} = \frac{m_{wet\ sed} * (1 - \%TS)}{\rho_{water}} \quad [13]$$

$$V_{Pore\ Water} = Volume\ of\ pore\ water\ (mL)$$

$$m_{wet\ sed} = mass\ of\ wet\ sediment (g)$$

$$\%TS = Percent\ total\ solids = = \frac{m_{dry\ sed} - m_{dish}}{m_{wet\ sed} - m_{dish}} \quad [14]$$

$$m_{dry\ sed} = mass\ of\ sediment\ after\ 12\ hours\ of\ drying\ at\ 106°C \text{ and pulversizing (g)}$$

$$m_{dish} = mass\ of\ weighing\ dish\ after\ ignition\ to\ remove\ organic\ mass\ (g)$$

$$\rho_{water} = density\ of\ water, assumed\ at\ 0.997\ g/mL$$

The pore water volume is then applied to the concentrations determined from HACH kits for ammonia and the ion chromatograph for nitrate, nitrite, and phosphate.

$$C_{Pore\ Water} = Sample\ Concentration * \frac{V_{Total}}{V_{Pore\ Water}} \quad [15]$$

$$Sample\ Concentration = Concentration\ measured\ by\ IC\ or\ HACH\ (\frac{mg}{L})$$

$$V_{Total} = Total\ unspiked\ serum\ bottle\ volume = 50\ mL + V_{Pore\ Water}\ (mL) \quad [16]$$

$$C_{Pore\ Water} = Pore\ Water\ Concentration\ (\frac{mg}{L})$$

Serum Bottle Tests for Rates

The serum bottle tests were conducted the day after sampling for each site. In each case, roughly 10 g of sediment were made into a slurry with 50 mL of deionized water in a serum bottle and left to react for twelve hours on the shaker at room temperature. Serum bottles were run in duplicate, and both spiked and unspiked serum bottle tests were performed. The target spike amount, 0.6 mg/L, was used for each trial. The tests were run open to air in order to maintain aerobic conditions, and the following equations were used to determine serum bottle nitrification rates:

$$m_{VS} = m_{wet\ sed} * \%\ TS * \%\ VS_{dry} = mass\ of\ volatile\ solids\ (g) \quad [17]$$

$$\%\ VS_{dry} = \frac{m_{dry\ sed} - m_{ign\ sed}}{m_{dry\ sed} - m_{dish}} = Percent\ volatile\ solids\ of\ dry\ sediment \quad [18]$$

$$m_{wet\ sed} = mass\ of\ wet\ sediment\ (g)$$

$$m_{ign\ sed} = Mass\ of\ sediment\ ignited\ at\ 550℃\ for\ one\ hour\ after\ drying\ (g)$$

$$C_{VS}\ \left(\frac{mg}{L}\right) = concentration\ of\ volatile\ solids = \frac{m_{VS}}{50\ mL + V_{pore\ water}} \times (\frac{1000\ mL}{L}) \quad [19]$$

$$Rate\ of\ Disappearance\ \left(\frac{mg\ NH4-N}{g\ VS*day}\right) = \frac{\left(\frac{dC_{serum}}{dt} * 24\frac{hours}{day}\right)}{C_{VS}} \quad [20]$$

$$\frac{dC_{serum}}{dt} = Change\ in\ nutrient\ concentration\ in\ serum\ bottle\ over\ time$$

$$reacted\ (\frac{mg}{L*hr})\ \ [21]$$

From here, the serum bottle rates were standardized to incorporate genomic data. Real-time PCR was conducted to quantify *amoA* present in each DNA sample. The amount of *amoA* can be directly correlated to the number of AOB present. To normalize serum rate by gene copy, Equation 22 was utilized.

$$\frac{\#\ amoA\ Gene\ Copies}{mg\ VS} = \frac{\#\ amoA\ Gene\ Copies}{m_{DNA\ Ext}*\%TS*\%VS} \quad [22]$$

$$\#\ amoA\ Gene\ Copies = Number\ of\ amoA\ copies\ reported\ using\ real\ time\ PCR$$

$$m_{DNA\ Ext} = Mass\ of\ sediment\ used\ in\ DNA\ Extraction\ (\text{mg})$$

After that, the genomic data was incorporated into the nitrification serum bottle results to help explain the nutrient flux data from each site. Nitrification rates per a*moA* gene were calculated for each site using Equation 23.

$$\frac{g\ NH4{-}N}{amoA\ Copy*day} = \frac{(\frac{dC_{serum}}{dt}*24\frac{hours}{day})}{C_{VS}*(\frac{\#\ amoA\ Gene\ Copies}{mg\ VS})} \quad [23]$$

Similar tests were run for denitrification, however, this time the serum bottles involved three trials. One trial tested carbon dependency by incorporating both acetate and nitrate, which was observed in comparison to a test involving just nitrate, and a third involved no spiking whatsoever. Each serum bottle was run in duplicate and in closed,

nitrogen-purged conditions. Spiking values were again 0.6 mg/L NO_3-N and 25 mg/L Carbon from acetate. The rates were calculated using the previous equations used for nitrification, while replacing ammonia for nitrate and *amoA* for *nirS*.

Nitrogen Mass Balance

The nitrification serum bottle tests were also used to calculate nitrogen mass balances for the sediment at the sites. Nitrification tests were chosen because these serum bottles likely displayed denitrification as well, due to less nitrate being present in solution after the serum bottle run time than stoichiometrically expected. Also, denitrification rates in serum bottle tests far outpaced nitrification rates, so it was no surprise that both could happen at the same time in the nitrification serum bottles. To determine the nitrogen mass balance, the masses of NH_3-N, NO_3-N, and NO_2-N were measured before and after the serum bottle tests. The difference between the summed amounts is simply the nitrogen mass balance of the sediment.

Task 4: Identify the Bacteria Participating in Nitrification and Denitrification Using Advanced Molecular Tools

DNA Extraction and Quantification

DNA was extracted from sediments within 24 hours of sampling using the PowerSoil (R) DNA isolation Kit 12888-50, MoBio Laboratories Inc. Duplicate DNA samples were extracted for each site. The procedure followed what was recommended by MoBio Laboratories and proper safety considerations were taken.

Concentration of DNA for each site was determined using a Nanodrop 2000 (Thermo, USA). This instrument used spectrophotometry to measure the DNA

concentration of samples as small as 1 μL. The sample port was rinsed with Milli-Q water before and after use and blanked with the proper eluent. To ensure proper quality control, the ratios of absorbance between multiple wavelengths was observed. The ratio of A260/A280 needed to be at around 1.8, while the ratio of A260/A230 was expected at 2.0-2.2. Accurate ratios of these absorbances corresponded to the purity of the DNA, and less pure DNA was not used for future analyses.

Functional Gene Identification and qPCR

Polymerase Chain Reactions (PCR) were carried out using a Mastercycler gradient (Eppendorf, USA) for the ammonia-oxidizing bacteria, denitrifying bacteria containing the nitrite reductase gene, anammox bacteria, and AOB. While different primers were used for every gene, mostly consistent master-mix blends of PCR reagents were used, sometimes the water amounts in each reaction were lessened or increased based on the DNA concentration of the sample DNA. Table 3.2 shows thermal cycler program and different functional genes used for PCR. Each 25 μl reaction mixture contained 12.5 μl of GoTaq, 0.5 μl of each of 0.2 μM forward and reverse primers, 1 μl DNA template, 0.5 μl of 10mg/mL of BSA, and the rest nuclease-free ultrapure water. PCR reactions follow a cycle of DNA denaturation, primer annealing, and primer extension. This means the double-stranded DNA becomes single-stranded in order for a primer to attach to gene-specific binding sites on the DNA, followed by the use of DNA polymerase to extend the DNA strand until the reverse primer is encountered on the DNA (Dorak, 2007). After running PCR, the resulting DNA was stored at -20°C to avoid degradation of DNA.

Table 3.2 Primers and PCR Programs for Target Genes

Metabolic Function	Gene	Primers Used	PCR Program	Reference
Nitrification	amoA (AOB)	amoA-1F (GGGGTTTCTACTGGTGGT)	95°C, 5 min; (95°C, 60 s; 56°C, 90 s; 72°C, 90 s) x 34; 72°C, 10 min	(Rotthauwe et al., 1997)
		amoA-2R (CCCCTCKGSAAAGCCTTCTTC)		
	amoA (AOA)	104F (GCAGGWGAYTACATYTTCTA)	95°C, 5 min; (95°C, 30 s; 57°C, 30 s; 72°C, 40 s; 80°C, 15 s) x 7 - touchdown 1°C each step until annealing temp = 51°C; (95°C, 30 s; 57°C, 30s; 72°C, 40s; 80°C, 15 s; 72°C, 40 s; 80°C, 15 s) x 40; 72°C, 5 min	(Francis et al., 2005)
		616R (GCCATCCABCKRTANGTCCA)		
Denitrification	nirS	cd3aF (GTCAACGTCAAGGAAACCGG)	95°C, 2 min; (95°C, 15 s; 60°C, 40 s; 72°C, 30 s) x 30; 72°C, 5 min	(Throback et al., 2004)
		R3cd (GACTTCGGATGCGTCTTGA)		
	nirK	F1aCu (TCATGGTGCTGCCGCGKGACGG)	95°C, 2 min; (95°C, 15 s; 63°C, 30 s; 72°C, 30 s) x 6 - touchdown 1°C each step until annealing temp = 51°C; (95°C, 15 s; 60°C, 30 s; 72°C, 30 s) x 30; 72°C, 5 min	(Throback et al., 2004)
		909R (GAACTTGCCGGTKGCCCAGAC)		

To test the identity of the PCR product, gel electrophoresis was carried out. This was run by creating 1% agarose gel in 1X TAE Buffer. After solidifying, the electrophoresis was run with gel submerged in TAE buffer at 80V for one hour. For every electrophoresis run, a DNA ladder was included to give an estimate of the base pair size for the PCR product. In addition, both a positive and negative control were run to indicate what both a successful and unsuccessful sample would look like. The positive control was extracted from a known pure culture of bacteria (for example, pure culture of nitrifier DNA was isolated to be used as a positive control). The genes used for positive control were also checked via sequencing to ensure that the gene was exactly what was expected. Negative control samples contained only mastermix with no DNA, therefore no band should appear after electrophoresis. If the sample DNA lined up correctly with the positive control and was roughly the fragment size expected based on the DNA ladder, the PCR was a success and more robust genomic analyses were carried out.

qPCR, or real-time quantitative PCR, evaluates the number of gene copies present in a sample by monitoring the fluorescence of the sample after every PCR cycle (Dorak, 2007). For each cycle, the temperature during which the fluorescence is measured increases, thus causing increasing amounts of double-stranded DNA to become single stranded (Dorak, 2007). Since the fluorescence is only emitted during the presence of double-stranded DNA, the fluorescence decreases as the temperature increases due to this forced denaturation (Dorak, 2007). A melting curve of temperature versus fluorescence can be created in this way.

Before running samples on the Thermalcycler qPCR, standards for each gene were created by measuring the melting curve for each gene based on a known starting gene copy amount. The melting curve for each sample was then compared to this calibration curve to

get a known gene copy for each sample. The following reagents were used for qPCR analysis.

- SYBR GREEN (light sensitive) - 10 μl
- Primer (Forward) 10 μM – 1 μl
- Primer (Reverse) 10 μM – 1 μl
- DNA Template – 2 μl
- Nuclease free water, Ultrapure – 6 μl

A mastermix was made for all components minus DNA and placed into a 96-well reaction plate. Samples were run in triplicate to avoid human error as pipetting in light-controlled areas can be difficult. qPCR programming was edited to reflect the same program used for PCR, with the addition of fluorescence measuring steps. The qPCR results were normalized for volatile solids in future analyses.

Gene Purification and Terminal Restricted Fragment Length Polymorphism (TRFLP) for Nitrifying Functional Genes

Both TRFLP and gene sequencing require purification of genes from gel electrophoresis to be used as the DNA source for analysis. To purify genes, a 75 μL PCR mixture of each sample was run and the product was verified via gel electrophoresis using just 3 μL of product. If the bands appeared bright enough and accurate, the rest of the 72 μL of samples were loaded on the gel for electrophoresis. The amplified PCR products of correct size were cut from the gel using an autoclaved blade. The products were extracted from the gel using a QIAGEN Gel Extraction Kit (QIAGEN, USA), which involved excising the gene band from the gel with a scalpel, dissolving the agarose from the sample,

and eluting the resuspended DNA from the resulting dissolved gel mixture. After eluting the DNA, the sample was checked again on the Nanodrop to ensure a high enough DNA concentration was liberated from the gel (the amount varied per analysis). The purified product can even be reloaded onto a gel to see if bands still appear after extraction.

TRFLP analysis was carried out for AOB in order to identify the species of *Nitrosomonas* bacteria present. This experiment required the same reagents as PCR, with the exception of using fluorescently labeled forward and reverse primers which otherwise have the same nucleotide sequence as nonlabeled primers. PCR was run for each site, followed by a gel purification. The resulting extracted genes were then digested with the restriction enzyme Taq 1. This restriction enzyme creates fluorescently labeled terminal restriction fragments; the size of each varies for each species of AOB. The University of Utah DNA Sequencing CORE facility ran the TRFLP experiments and were able to identify the species of AOB present based on these resulting DNA fragments. The resulting TRFLP electropherogram can be analyzed to identify species of bacteria present in sample.

Cloning and Sequencing

In addition to determining the nucleotide sequence of the positive controls used for gene identification, sequencing was carried out for the three sites which displayed the highest rates of nitrification during the sampling events: Unit 2, Unit 1, and South Area. Cloning is a four-step process starting with gel extraction; the fifth step is essentially a sequencing. Again, 75 μL of sample was run on PCR to obtain a higher purified gene concentration. The first step of cloning includes the ligation step, where the gene of interest is inserted into a plasmid vector for incorporation into *E. coli* bacteria. Vectors and host *E. coli* cells were obtained from the TOPO TA cloning kit/TOP10 (Invitrogen) and very careful, exact procedures

were carried out to ensure sufficient clones were created. After creation of a plasmid vector via ligation, the next step of cloning included transferring the vectors into the host cells through a process known as transformation. The procedure included inserting the vectors into a cell batch and growing the cells overnight on sterile petri dishes with LB broth/agar growth media. A concentration of 50 μg kanamycin/mL was prepared in the growth media to ensure the cells would grow properly. Three plates were used per site and the plates were incubated overnight. Last, cultivating colony cultures of the bacteria rounded out the cloning sequence. After 16 hours of growth, bacterial spores appeared on the plates; these could be picked out with a toothpick for the next growth step. This involved again growing cells overnight—this time the cells were grown in liquid LB broth with the same kanamycin concentration. The resulting tubes displayed a cloudy bacterial growth, meaning they were ready for plasmid extraction and sequencing.

The plasmid extraction step was carried out using a Zyppy Plasmid Miniprep Kit (Zymo Research, USA). Fifteen clones were picked per site, and after the plasmid extraction occurred, the DNA concentration was checked for the extractions. If the plasmid DNA showed lower than 40 ng/μL, the plasmid extraction was repeated for a site. Once every plasmid extraction showed a high enough concentration, the clone was prepared for sequencing. This included adding the M13 Forward primer and arranging it on a 96-well sequencing tray. The samples were sent for analysis to the University of Utah DNA Sequencing CORE facility. Resulting sequences were used to form a phylogenetic tree of nitrifiers for the target sites. Phylogenetic trees were created for nitrification and denitrification bacteria; however, the same could be done with any specialized types of bacteria, including DNRA bacteria and any annamox bacteria on site. Identifying these bacteria was outside the scope of this project but could be an interesting addition.

Task 5: Data Analysis Using Statistical Software "R"

Z-Tests

Based on the central limit theorem, the arithmetic mean of a set of variables should display a normal distribution. If a data point falls outside the sample distribution, it is not representative of the sample set (Montgomery et al., 2011). Z-tests are based on this assumption and the normal distribution for Z-tests are displayed in Figure 3.6. A Z-score is calculated by the following equation (Montgomery et al., 2011)

$$Z = \frac{\bar{X} - \mu}{s} \quad [24]$$

$$Z = Z - score$$

$$\bar{X} = sample\ value$$

$$\mu = mean\ of\ data\ set$$

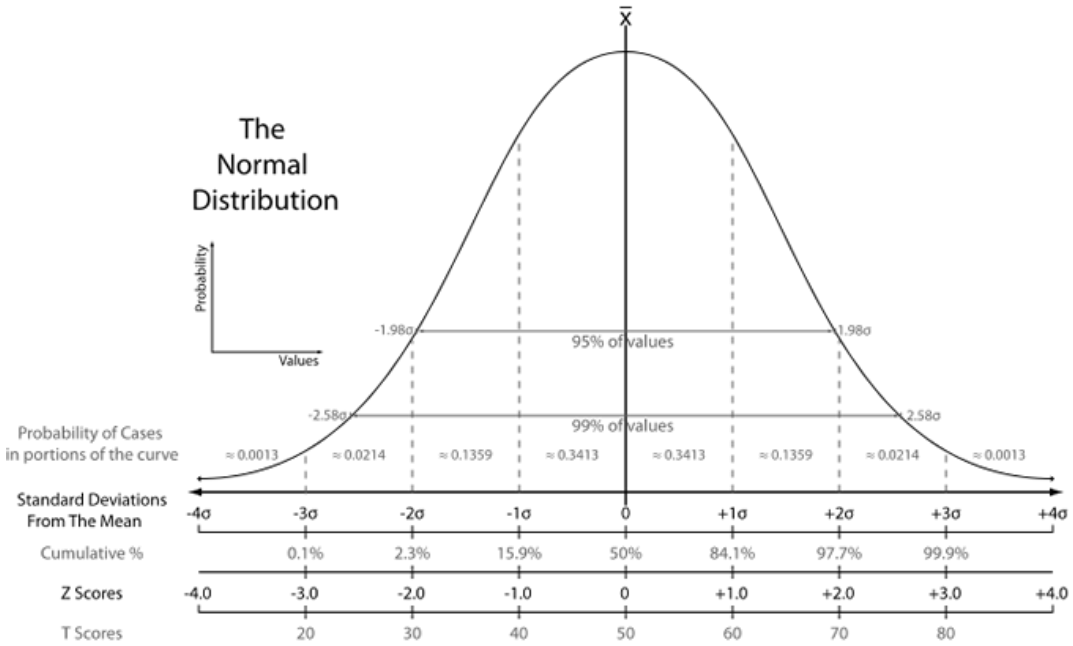

Figure 3.6 Z-test normal distribution (A'Design Award and Competition, 2015)

Based on Figure 3.6, Z-scores are indicative of what percentage of the data set are predicted to have the same value. For example, a sample value's calculated Z-score of zero corresponds to roughly 34% of the variables in a data set, and half of the data set has values above this sample value. This can be a powerful tool as any variables which lie on the extreme ends of the bell curve (Z-score of -4 or 4) have only a 1% chance of being within the sample mean.

Z-tests were carried out for all sediment flux values to ensure that all of this data set can be used for more advanced statistical analyses. The tests were carried out in Excel as a form of statistical diagnostics.

Identify Which Factors Could Contribute to the Variance

The same method for Z-tests were carried out for all variables collected during the experiment to account for any sediment fluxes which did not correspond to the overall sample set. For example, any sites which showed a sediment ammonia flux outside of the bell curve could have more Z-tests carried out on other individual variables for the sites which may have been a factor in ammonia sediment flux.

Principal Component Analysis on Ammonia, Nitrate, and Phosphate Sediment Fluxes to Determine Which Variables Have the Most Impact on These Fluxes

To test for variable correlation, a principal component analysis incorporating a biplot representation of variable relationships was used. R statistical software was used for all PCA statistical analyses. A principal component analysis creates new variables which are linear combinations of the original variables in a study (Ringner, 2008). For example, in this case,

principal components were created by combining variables such as ambient pH, nutrient concentration, and amount of bacteria present in sediment. The two principal components which are the least correlated but show the highest variation between original variables are plotted against each other to create a PCA chart (Ringner, 2008). This creates the backdrop for which each individual original variable can be plotted against—incorporation of the original variables onto the PCA chart creates a biplot. The original variables are plotted as vectors on the PCA chart and the size and angle of the vectors depends on how each variable was factored into the PCA variables. As a result, any vectors which form acute angles are more correlated than vectors which form large angles (Gabriel, 1980). Therefore, biplots were used as a visual reference to determine which variables are correlated to each other. All PCA functions were carried out in R Studio, all data was imported over the R Studio interface.

PCA requires more observations than variables, but in the case of this study, there were often more variables than observations. In this case, two separate biplots were created for the same sample set and only variables most correlated to sediment flux from each biplot were included in a final biplot. The end results for which variables are the most correlated to sediment flux were observed from this final biplot.

Quality Control Summary

Chamber Installation and Field Sampling

Care was taken as not to disturb sediments when installing chambers. To check that the installation went well and to check for reproducibility, the chambers were installed in duplicate and sampled soon after installation. Elevated levels of turbidity indicate that the sediments were disturbed during installation. The flux rates should be compared between

duplicates, and if previous data suggests that the site has low nutrient concentrations, lengthen the residence time for the experiment.

Sampling field blanks were used over the course of the project to ensure that no in situ activities were contributing to any nutrient concentrations in the samples. One field blank was required per site. The field blank included only Milli-Q ultrapure deionized water and the before and after sampling concentration of the water was measured. The field blank was open and closed on the sampling canoe of each site, filtered through the on-site filtering apparatus, and measured back at the lab. Two other field blanks of low and high concentration (low field blank: 0.1 mg/L NO_2-N, 0.5 mg/L NO_3-N, 0.1 mg/L PO_4-P, and 0.5 NH_4-N; high field blank: 0.5 mg/L NO_2-N, 2.5 mg/L NO_3-N, 0.5 mg/L PO_4-P, and 2.5 mg/L NH_4-N) were included as well to bring field blank concentrations to detection range.

To limit disturbances by wind or other weather activities, only sunny or partly cloudy days with low wind were chosen for sampling. Any site conditions which led to unrepresentative samples were minimized and recorded.

Ion Chromatography

Proof of correct performance and accurate calibration curves were the first steps in the QA/QC of ion chromatography. A calibration curve was created every six months, and prior to extended use the curve was tested with known concentrations of reagents (PO_4, NO_3, and NO_2) to prove the curve to be accurate. If the results deviated from expected by +/- 10%, this indicated a problem with the IC or calibration curve.

After confirming that the IC and calibration curve were operating correctly, a laboratory fortified blank (LFB) and laboratory reagent blank (LRB) were tested along with every other sample during a batch test. The LFB included a known concentration of

nutrients, while the LRB contained deionized water. It is in good practice to include two LRB samples before and after the samples being analyzed by the IC, as this helps clean the equipment as well as providing blanks at the beginning and end of each sample batch. One LFB was also added at the beginning and end of each sample batch. Also, one out of every ten samples included an LFB, again to measure the dependability of the calibration curve and IC. Again, the percent error of expected concentrations of the LFB had to remain within +/- 10% of the expected value. LRB samples were used to ensure that ion chromatograph error was not contributing to increases in measured concentrations. In addition, results from multiple concentrations of LRBs were used to quantify the lower detection limit of PO_4, NO_3, and NO_2 concentrations in the IC. The standard deviation plus average concentration of all 200+ LRB measurements was identified as the lower detection limit of the ion chromatogaph.

To prove that spiking did not have any matrix effects, a laboratory fortified sample matrix (LFM) was used. This was a duplicate of the aliquot previously added to deionized water, this time added to ambient water from the sample site. As long as the percent recovery of the LFM was 90-110%, the calibration curve and instrument was still accurate.

Serum Bottle Tests

All samples were run in duplicate to prove reproducibility. All results were reported in nitrification or denitrification rate per amount of gene present. For each site, a negative control was included, which entailed adding a nitrification or denitrification inhibitor. The nitrification inhibitor chosen was allyl thiosulphate at 50 mg/L, while denitrification was inhibited through constant aeration of the slurry throughout the experiment.

DNA Extraction and Quantification

All DNA extraction and quantification was done in duplicate to measure reproducibility. In addition, the Nanodrop used for quantification had its own QA/QC measurements that are displayed with every reading. The A260/A280 ratio must be close to 1.8 and the A260/A230 ratio must be close to 2 in order to prove the quantification was carried out correctly. In addition, measuring blank samples (comprised of the eluent from the last step of DNA extraction) can be a good way to show that the Nanodrop is being used correctly.

Total Organic Carbon

This QA/QC was similar to ion chromatography. One should always create one's own calibration curve and test the curve every six months. Standard deviation and percent error were tested using the exact same method as ion chromatography.

CHAPTER 4

RESULTS AND DISCUSSION

Task 1: Design and Fabricate In Situ Flux Chambers and Flux Measurements

In situ chambers were fabricated in the lab and were tested for water tightness by filling the control chamber with tap water in the lab. A practice run was also conducted in the vicinity of one of the sites to ensure all quality checks.

QA/QC

Several quality control methods were carried out for laboratory experiments and field sampling activities. First of all, with every operation of the IC, a calibration curve was created to account for any slight changes in IC running conditions, such as conductivity of operation. After accounting for these deviations, the field blanks, LFM, LRB, and LFB, were considered.

The average percent error (AVG) and standard deviation (STD) were measured for field blanks from sampling events as well as the low and high concentration field blanks. The average and standard deviation were calculated from both sampling events at nine sites and are shown in Table 4.1. The average percent error and standard deviation were kept below 10% each, as required for collaboration with the USEPA.

Similarly, LFM samples were taken during every sampling event for each site. The AVG and STD are shown in Table 4.2 for all sampling events.

Table 4.1 Field Blank QA/QC

Field Blank IC QA/QC All Sites				
	NO_2-N	NO_3-N	PO_4-P	NH_3-N
AVG	2.5%	3.1%	7.6%	4.4%
STD	2.2%	7.8%	9.0%	6.7%

Table 4.2 LFM QA/QC

LFM IC QA/QC All Sites			
	NO_3-N	PO_4-P	NH_3-N
AVG	6.8%	14.1%	7.3%
STD	6.5%	17.1%	5.8%

Unfortunately, the sub-10% requirement for the AVG and STD of all LFMs from sampling was not fulfilled for phosphate spiking. Upon further investigation, both NE Pond and Bear River Unit 5C had much higher percent errors from expected spiking concentrations than any other site, as shown below (DUP stands for duplicate LFM samples). The expected phosphate concentrations were to be 0.1 mg/L PO_4-P higher than ambient concentrations of phosphate from each site. When the percent error for the LFM was higher than 10% for NE Pond and Bear River Unit 5C, an in-lab experiment was carried out to determine what could be causing the discrepancy in results. These results are displayed in Table 4.3. The results of this table could be used for justification of further research in this area.

Table 4.3 LFM Result Deviation Test

Sampling ID	Date	Expected PO_4-P (mg/L)	Measured PO_4-P (mg/L)	Percent Error
NE Pond Site Ambient	5/22/2014	-	0.008	-
NE Pond Site LFM	5/22/2014	0.108	0.074	31.5%
NE Pond Site LFM DUP	5/22/2014	0.108	0.073	32.4%
NE Pond Site Ambient	8/25/2014	-	0.004	-
NE Pond Site LFM	8/25/2014	0.104	0.036	65.4%
NE Pond Site LFM DUP	8/25/2014	0.104	0.039	62.5%
Bear River Unit 5C Ambient	6/30/2014	-	0.007	-
Bear River Unit 5C LFM	6/30/2014	0.107	0.090	15.9%
Bear River Unit 5C LFM DUP	6/30/2014	0.107	0.083	22.4%
Bear River Unit 5C Ambient	9/17/2014	-	0.021	-
Bear River Unit 5C LFM	9/17/2014	0.121	0.074	38.8%
Bear River Unit 5C LFM DUP	9/17/2014	0.121	0.070	41.9%

To explain the large percent error in expected phosphate concentrations for LFM samples for this site, a separate matrix-spiking experiment was carried out. The results showed lower percent errors in LFM expected concentrations when the pH of the LFM samples were lowered before spiking, indicating that some kind of chelating matrix was occurring with phosphate and possibly some dissolved species in the water when spiking. Perhaps as a result, NE Pond and Bear River Unit 5C displayed the lowest average ambient

phosphate concentrations of all nine sites for the sampling events. This is similar to the sediment interactions with phosphate—when the pH is dropped, phosphorus attached to calcium in sediment is dissolved into the WC. It is possible that a similar dissolved species is absorbing phosphorus in the water at NE Pond and Bear River Unit 5C, so the phosphorus fluxes for sediments at these sites were reported but not included in statistical analyses. The LFM QA/QC results showed a more acceptable AVG and STD when these sites were not included. Table 4.4 lists the adjusted LFM QA/QC results.

While field blank and LFM QA/QC practices were carried out only for analyzing sample events, LRB and LFB samples were included for every run of the IC. As a result, over 200 LRB and LFB results were collected to monitor IC performance. The LRB results are shown in Table 4.5. Again, the STD had to remain below 10% each in order to maintain acceptable IC performance. Quality control results for ion chromatograph operation were used as justification for repairs. When reproducibility became worse, technicians were hired to maintain proper ion chromatograph operation. Operational standards from HACH would be used to further test the machine once it becomes functional again. The calibration curves of the ion chromatograph were also tested after these instances.

Table 4.4 Adjusted LFM QA/QC

	LFM IC QA/QC Adjusted*		
	NO_3-N	PO_4-P	NH_3-N
AVG	**6.8%**	5.9%	7.3%
STD	**6.5%**	4.2%	5.8%

*Excludes PO_4-P LFM samples for NE Pond and Bear River Unit 5C

Table 4.5 LRB QA/QC

LRB IC QA/QC			
	NO_2-N	NO_3-N	PO_4-P
Average Conc (mg/L)	0.001	0.001	0.003
STD	0.002	0.004	0.003

Instead of average percent error, the average concentrations of all LRB samples were monitored. The average concentration plus standard deviation provided a detection limit for usage of the ion chromatograph. Last, laboratory fortified blank samples were used to ensure the ion chromatograph was displaying correct concentrations for all measurements. These results are given in Table 4.6. Since the average percent error and standard deviation of the 100+ laboratory fortified blank samples stayed below 10% for both sampling events, the ion chromatograph operation was a success.

The operation of the total organic carbon analyzer followed similar quality assurance/quality control procedures. The machine was calibrating using a calibration curve before every use and check standards had to show average percent error and standard deviation below 10% each. These results are in Table 4.7. If the results were not within the 10% range for a site – the site was repeated. This occurred once during the project and all information for the sampling event was discarded and repeated within a week. The identified error for that sampling event was operation error in the amount spiked for the laboratory fortified blank, which is shown in Table 4.6. Superior quality control was required for this project as it was in collaboration with the Environmental Protection Agency.

Table 4.6 LFB QA/QC

IC LFB QA/QC			
	NO_2-N	NO_3-N	PO_4-P
AVG	8.8%	7.3%	5.2%
STD	5.6%	6.6%	4.3%

Table 4.7 npDOC Check Standard QA/QC

npDOC Check Standard	
AVG	2.8%
STD	4.1%

Ambient Nutrient Concentrations

Two rounds of sampling events were conducted in 2014, the first in May and the second in August, to record nutrient dynamics in early and late summer (early fall). Table 4.8 shows the ambient concentrations of targeted nutrients at all 9 sites. These ambient nutrient samples were taken as grab samples at the beginning of the sampling event. It should be recognized that the high-concentration sites in the Farmington Bay Duck Club selected for this project included FBS and Unit 2, the mid-range concentration sites were Unit 1, Turpin, and FB SE Unit 1, and the low-concentration sites were FB NE Pond and South Area. Ambassador Duck Club Pond #1 and Bear River Nature Preserve Unit 5C both had mid- to low-range nutrient concentrations and were included to compare against Farmington Bay Wildlife Management Area sites.

Table 4.8 Ambient Nutrient Concentrations (mg/L) in Water Column Measured at Each Site

	NO_3-N		**PO_4-P**		**NH_3-N**	
Site	May Sampling	August Sampling	May Sampling	August Sampling	May Sampling	August Sampling
FBS	2.48	0.71	0.419	UDL	0.194	0.127
FB SE Unit 1	0.023	UDL	0.048	0.121	1.27	0.03
Turpin	0.009	0.009	0.09	0.078	UDL	0.081
South Area	UDL	0.008	0.386	1.127	UDL	0.029
FB NE Pond	0.184	0.041	0.008	UDL	0.038	0.021
Unit 2	0.574	0.958	0.578	0.494	0.821	0.379
Unit 1	UDL	UDL	0.717	0.1	UDL	0.016
Ambassador	0.063	0.496	0.283	0.451	0.033	0.055
Bear River	UDL	0.029	0.007	0.022	0.025	0.05

***UDL (Under Detection Limit) for PO_4-P: < 0.006 mg/L, UDL for NO_3-N: < 0.006 mg/L, UDL for NH_3-N: < 0.015 mg/L**

Since nitrite nitrogen (NO_2-N) was under the detection limit (UDL) at most of the sites, it was not included in Table 4.8 - Ambient Nutrient Concentrations (mg/L) in Water Column Measured at Each Site. Low NO_2-N at sites implies either complete nitrification of ammonium to nitrate and/or, perhaps, reduction of NO_2-N to reduced forms of nitrogen. Nitrite oxidizes quickly to nitrate in most cases, so potentially, the nitrite of some samples could have undergone oxidation before analyzation using ion chromatography.

Pore Water Nutrient Concentrations

Pore water nutrient concentrations are important because if nitrification and denitrification are occurring in sediments, the substrates for these reactions could be coming from the pore water. The possibility of concentration gradients controlling the flux of nutrients could be high, so any difference in ambient and pore water concentrations could be important. The pore water nutrient concentration results are shown in Table 4.9. The pore water concentrations of nitrate decreased between the sampling events, while the ammonia pore water concentration overall increased. The changes in these pore water concentrations could be explained by identifying the factors controlling sediment flux.

Table 4.9 Pore Water Nutrient Concentrations (mg/L) Measured at Each Site

	NO_3-N		**PO_4-P**		**NH_3-N**	
Site	May Sampling	August Sampling	May Sampling	August Sampling	May Sampling	August Sampling
FBS	0.71	0.47	2.98	5.03	1.63	3.91
FB SE Unit 1	0.46	0.55	3.14	8.44	0.193	1.07
Turpin	0.79	0.49	1.56	3.25	0.331	0.421
South Area	0.31	0.25	2.46	1.66	2.96	2.51
FB NE Pond	1.05	0.36	2.98	2.56	0.792	0.039
Unit 2	0.26	0.29	3.54	4.68	1.23	1.4
Unit 1	0.88	0.41	1.5	2.65	2.81	1.68
Ambassador	0.32	0.39	2.12	9.32	2.6	4.39
Bear River	0.46	0.36	1.63	2.71	1.4	0.587

Ammonia Sediment Flux

Figure 4.1 depicts the ammonia flux of each site side by side for all conditions and sampling dates. Ammonia sediment flux results which fall below the minimum detection limit of ±0.02 g NH_3-N/m^2/day were replaced with a value of zero.

Unspiked sediment flux results included the first four hours of sampling, which monitored nutrient concentration changes in the WC and SED after being filled with ambient water.

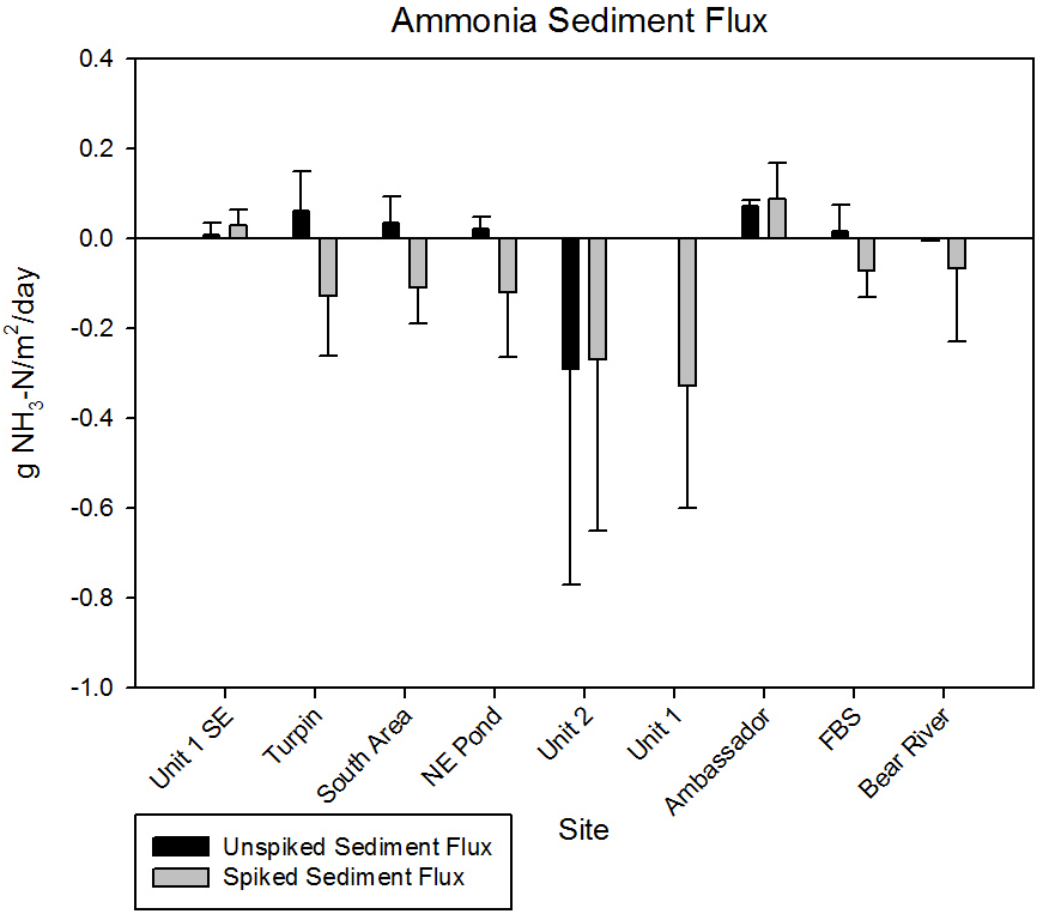

Figure 4.1 Ammonia sediment flux for all of the sampling sites in two events of early and late summer

The chambers were spiked to add 0.5 mg/L NH_4-N, 0.5 mg/L NO_3-N, and 0.1 mg/L PO_4-P to the WC and SED to allow any nutrient concentrations below detection limit to become observable and to show any reactions to a nutrient pulse. Nitrogen concentrations for spiking were higher than phosphorus concentrations to stimulate any nitrifying and denitrifying bacterial responses. The early and late summer sampling events were averaged together because a t-test of the results displayed a P value of 0.261 and 0.622 for unspiked and spiked nutrient fluxes, respectively. Another t-test comparing the spiked and unspiked sample means did, in fact, show a statistically significant difference, with a P value of 0.033. During unspiked conditions, nearly all sites contained sediment that was a source for ammonia, all with the dramatic exception of Unit 2 during May, which showed indications of being an ammonia sink. However, during August Unit 2 returned to having sediment as an ammonia source. During spiked conditions, on the other hand, sediment became mostly a sink for ammonia—possibly as a result of providing extra bioavailable nitrogen for nitrification. Due to the more positive ammonia sediment flux during unspiked conditions, it is suggested that ammonification of soil organic matter to ammonia outweighs nitrification processes (Kadlec et. al, 2009). Positive ammonia flux in this case indicates that the rate of ammonification is higher than the rate of nitrification in regard to ambient ammonia concentration.

The ammonification rate itself is hard to quantify, unfortunately, because it depends on the composition of organic matter undergoing decomposition, but one approximation of ammonification rate is 0.004 – 0.357 g N m^{-2} d^{-1} (Reddy, 2008; deBusk et al., 2001). By comparison, nitrification rates of sediments in other studies have shown a value of 0.01 to 0.161 g N m^{-2} day^{-1} (deBusk et al., 2001). Due to the higher values of ammonification than nitrification, the ammonia flux of sediment may contribute to eutrophication. In a separate

study of a eutrophic lake by Hohener, benthic flux chambers recorded an ammonium flux of 1.1-16.1 mmoles NH_4 m^{-2} d^{-1}, or, to compare with this study, 0.0154 – 0.225 g NH_4-N m^{-2} d^{-1} during the course of the Hohener study (Hohener et al., 1994). The range for this Utah wetland study was broad; it displayed an ammonia flux range of -0.563 to 0.254 g NH_3-N m^{-2} d^{-1} during unspiked conditions. However, both extremes were displayed by Unit 2. Factoring out Unit 2 would result in an ammonia sediment flux range of 0 – 0.122 g NH_3-N m^{-2} d^{-1}. Therefore, half of the sampling events showed ambient ammonia sediment fluxes that fall within other literature values for ammonia sediment flux in eutrophic water bodies.

Comparing the spiked sediment flux for ammonia to eutrophic lake sediment flux values is a wasted effort, as spiking the sample chambers showed an ammonia sediment flux rate closer to the isolated rate of nitrification from the deBusk review without consideration of ammonification (deBusk et al., 2001). Conditions explaining the sediment's high capability to consume ammonia during spiking could further be explained by statistical analysis of contributing factors to sediment flux. As for SAV response, a variety of plants have been shown to display a higher tolerance to elevated (below 50 mg/L) ammonia surface water concentrations—this is understandable, as ammonia sediment fluxes are typically in the positive range (Clarke et al., 2002).

Nitrate Sediment Flux

Nitrate sediment fluxes of the sites, when displayed side by side, show a more consistent trend than with ammonia. In the case of this project, nitrate fluxes were considered instead of nitrite fluxes because of the rapid oxidation of nitrite to nitrate that occurs in wetland environments, which causes the resulting historical trend of nitrite concentrations in ambient water being far lower than nitrate concentrations (*Report on the*

environment, 2009; Wetzel, 2001). Due to this rapid oxidation to nitrate, chambers were not spiked with nitrite (also, many sites showed very low concentrations of nitrite). Preliminary nitrite sediment flux measurements also showed no sites with a statistically significant nitrite sediment flux trend. The nitrate in the wetlands is likely being removed here due to denitrification, and this process readily happens due to the large diversity of microorganisms that take part in denitrification (Kadlec, 2009). Similar to ammonia fluxes, Figure 4.2 gives a visual representation of the changes in spiked and unspiked nitrate fluxes for each site during both sampling events. Also similar to ammonia flux, the P values of the early and late summer sampling events were calculated as 0.291 and 0.752 for unspiked and spiked sampling, showing that, again, the two events must be averaged. After averaging, spiked and unspiked nitrate fluxes showed a P value of 0.02 during a t-test, proving significant difference in the sample populations. Nitrate sediment flux results which fall below the minimum detection limit of ± 0.03 g NO_3-N/m^2/day were replaced with a value of zero.

Unspiked sediment nitrate flux results suggested the sediment behaved similarly to previously observed sediment from eutrophic lakes as, Hohener observed a nitrate sediment flux of -0.034 to -0.155 g NO_3-N m^{-2} d^{-1} in his eutrophic wetland study (Hohener et al., 1994). In fact, only one site—Unit 2—displayed nitrate sediment fluxes at a higher removal rate than those observed in the eutrophic study. Some sampling events even displayed sediment as being a source for nitrate; this is an unfortunate observation as elevated levels of nitrate in the surface water have been proven to have a harmful effect on drupelets, the reproductive structure for SAV in the GSL wetlands (Carling et al., 2013). In the case of sites that display sediment as being a source of nitrate, this could indicate that nitrification is outpacing denitrification or not enough organic carbon is bioavailable for denitrification in the sediment.

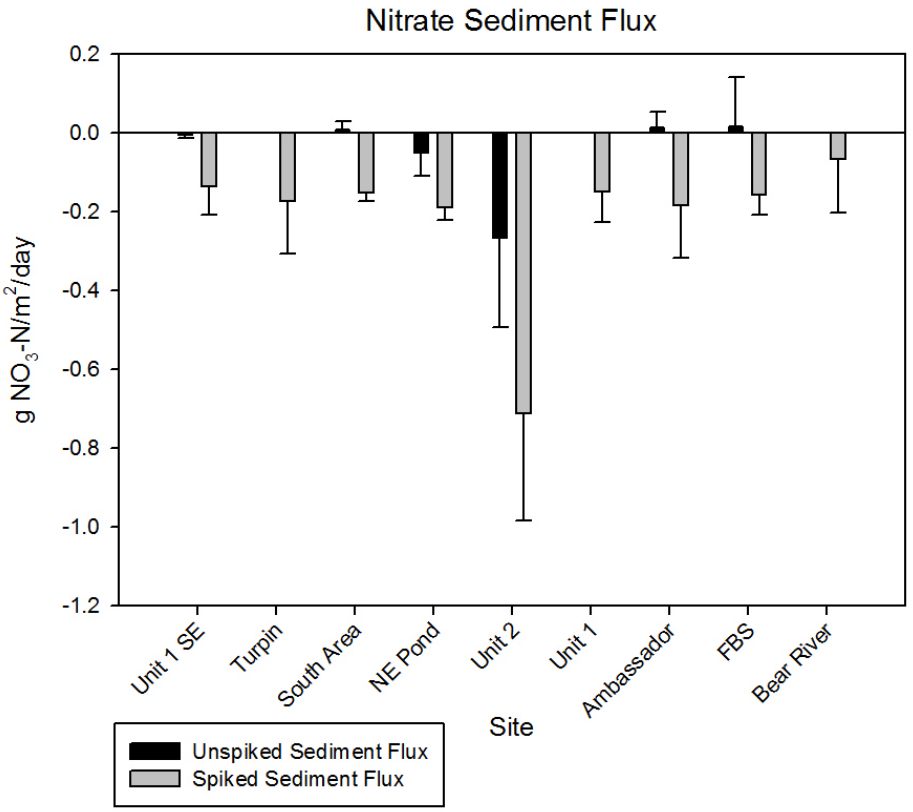

Figure 4.2 Nitrate sediment flux for all of the sampling sites in two events of early and late summer

The ability of sediment in eutrophic water bodies to remove nitrate could be explained by the high observed rate of denitrification in wetlands. Other studies reflect a denitrification rate in wetland sediments of 0.003 – 1.02 g N m^{-2} day^{-1}, a much higher range than nitrification rates possibly due to the larger bacterial community that is involved in this heterotrophic process (deBusk et al., 2001). This is a robust estimation. Even after spiking, nitrate sediment flux results did not outpace the observed wetland denitrification rate. Serum bottle tests to observe denitrification in this study confirm high denitrification rates, as well. By definition, denitrification does not occur in the aerobic zone of the water column, so the observation of sediment being a sink for nitrate corresponds well to other literature, field observations, and to the science of nitrogen cycling.

Phosphate Sediment Flux

The phosphate sediment fluxes (Figure 4.3) showed a general trend of the sediment being a sink for phosphate for nearly every nonspiked condition. Phosphate sediment fluxes again showed no significant difference for early and late summer sampling (P value of 0.572 and 0.844 from t-tests of unspiked and spiked sediment flux). Unlike nitrate or ammonia fluxes, however, phosphorus sediment fluxes showed less significant difference between spiked and unspiked sampling conditions with a t-test P value of 0.711. Despite the lack of significant difference between spiked and unspiked results, these were left as distinct sample populations to be able to compare to the ammonia and nitrate sediment flux results. Phosphate sediment flux results which fall below the minimum detection limit of ±0.02 g NH_3-N/m^2/day were replaced with a value of zero.

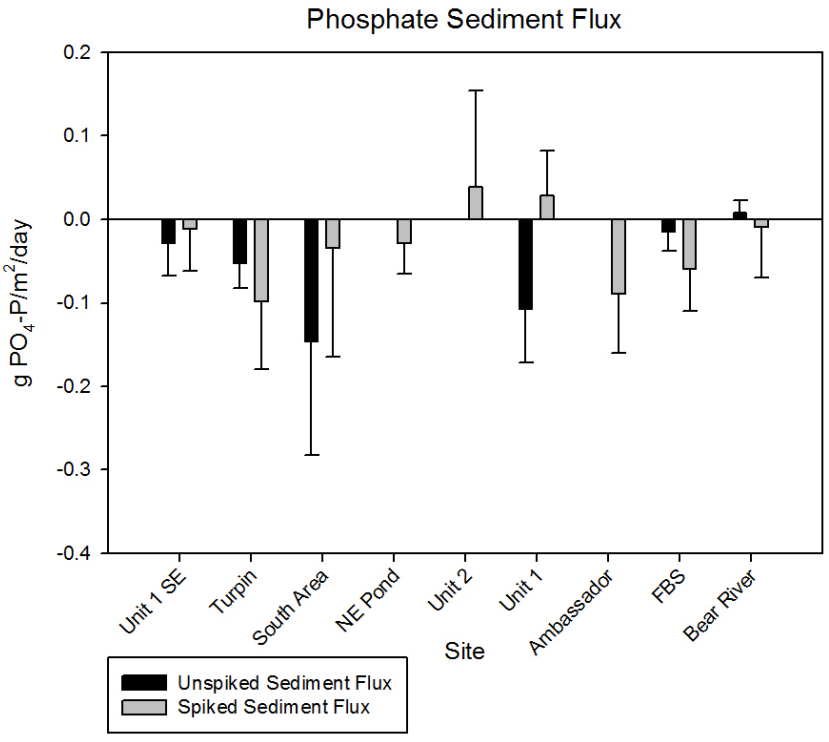

Figure 4.3 Phosphate sediment flux for all of the sampling sites in two events of early and late summer

Due to the variety of physical, chemical, and biological processes which add or remove phosphorus from sediment (Wetzel, 2001), it is hard to relate the phosphorus fluxes between different wetland sediments. For example, one study found sedimentation rates of phosphorus to be roughly 0.010 g PO_4-P m^{-2} day^{-1} (Reddy, 1999), but this number quantifies only part of the overall process. The same study describes organic soil as an overall sink at a rate of 0.04 to 1.1 g PO_4-P m^{-2} day^{-1} (Reddy, 1999). While a similar approximation of the average rate of phosphorus disappearance for the nine sites of this study equates to 0.0620 g PO_4-P m^{-2} day^{-1} based on changes in ambient phosphate concentration over time between the two sampling events. Possibly to account for this variation in phosphate sediment fluxes by site, a study of phosphate fluxes in shallow, eutrophic lakes observed a range of 0.015–0.1 g P m^{-2} day^{-1} (Jenson et al., 1992). One unspiked observation (Bear River Unit 5C in late summer) showed phosphorus release in this range, while all other sampling events displayed phosphorus release from sediment during the daytime at a level below those of the observed eutrophic lakes. Spiking caused many sites to show sediment as a phosphorus source, however. Like nitrate, phosphorus release from the sediment also has a proven poor impact on SAV health (Carling et al., 2013).

Conclusions for Task 1

Overall, measuring the sediment fluxes at each site during the sampling events gave a dependent variable with which to compare other variables during the statistical analysis. Several key points were noted for this task:

- The sediment was overall a sink for all spiked variables, with exceptions.
- Unit 2 sediment fluxes displayed the largest extremes in rate of nitrogen removal or addition.

- Half of the sampling events showed unspiked ammonia sediment fluxes to be close to those observed in eutrophic wetlands, while almost every sampling event showed unspiked nitrate sediment fluxes similar to those observed in eutrophic wetlands.
- Nitrate sediment fluxes on the spectrum observed in eutrophic wetlands could harm SAV health—if phosphate sediment fluxes were to become more positive it would also harm SAV health.

Task 2: Conduct P Speciation Using a Sequential Extraction Technique and Determine Other Parameters in Sediment Core Samples

The species of phosphorus present are important because only loosely bound phosphorus is bioavailable, while other forms of phosphorus can be released by pH fluctuations. In addition, the soil can act like activated carbon for phosphorus, allowing adsorption onto available sites. The results from phosphorus speciation studies are shown in Figure 4.4. Only sediments from the early summer sediment sampling for Farmington Bay wetlands were analyzed for phosphorus speciation due to the time dependence of this analysis (Ambassador Duck Club and Bear River Nature Reserve were added to this study slightly later than the Farmington Bay sites). However, it was speculated that the phosphorus content of the sediment remained in the same ratios during the late Summer sampling event as the early Summer sampling event. This assumption was based on the short (several month) timeframe between sampling events. It could be a worthy research opportunity to determine the rate of changes between the species of phosphorus in wetland sediment and how this may apply to further regulation of phosphorus levels entering the wetlands.

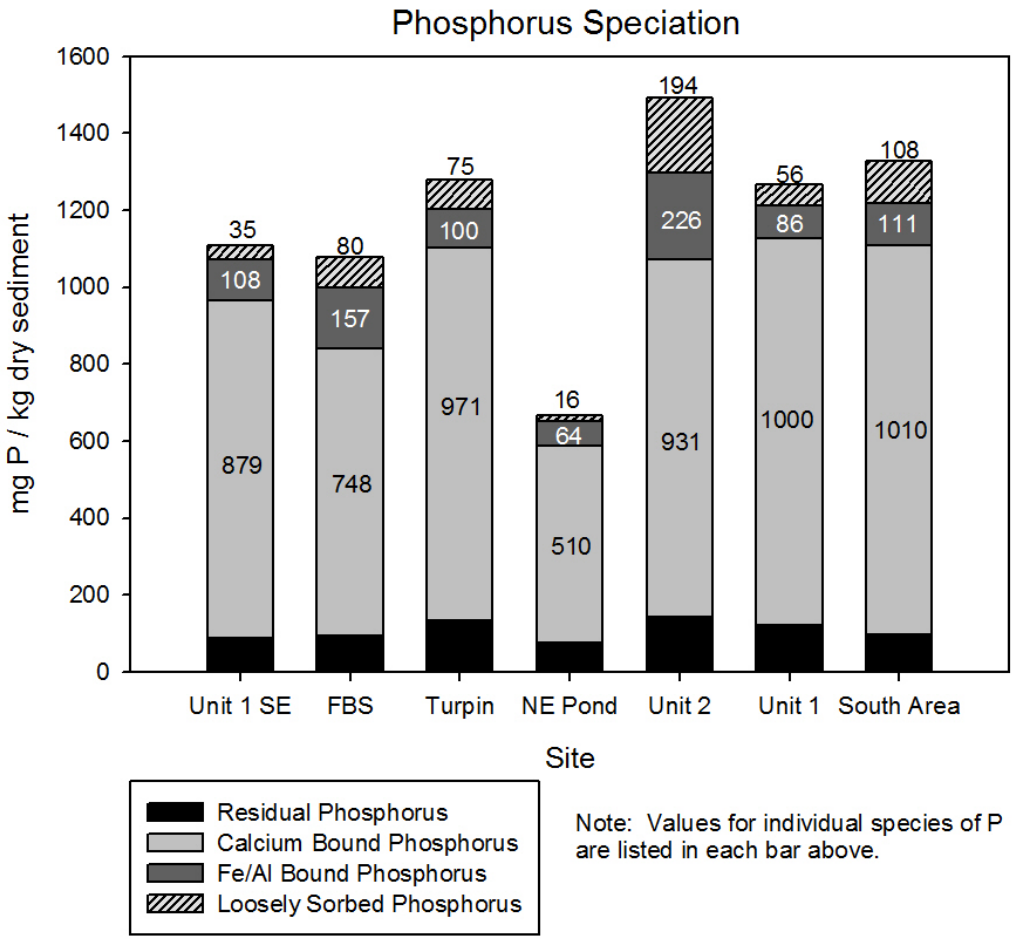

Figure 4.4 Phosphorus speciation results

As for the compared sites, Unit 2 showed the most total phosphorus, but this could be the result of having a higher volatile solids percentage with bioavailable loosely bound phosphorus. On the other hand, NE Pond portrayed very low phosphorus in sediments. This corresponds well to the interesting disappearance of phosphorus from the water column at NE Pond due to complex formation. The most prevalent form of phosphorus in the sediment was attached to calcium; an average of 74% of the phosphorus in the sediments at each site was bound to calcium. This could be a result of the amount of clay in the sediment. This portion of phosphorus in the sediment, as well as residual phosphorus, is

mostly immobile; this is due to the fact that acidic water conditions liberate calcium-bound phosphorus. Since the pH of the ambient water remained above 7 for the entire sampling event, the phosphorus in sediment attached to calcium may rarely be released. Overall, there is no obvious visible trend between the phosphorus sediment flux and any species of phosphorus in the sediment. Advanced statistical correlation techniques may be required to relate sediment flux values with speciation results.

Regardless, the average mass ratio of organic nitrogen to organic phosphorus in sediment (loosely bound) is 74:1, which is similar to other recorded values of 76:1 to 81:1 (Craft et al., 2002). Again, the high retention time of the wetlands accounts for higher amounts of phosphorus in the sediments. The phosphorus speciation experiments were run in conjunction with a total phosphorus experiment using the perchloric acid digestion method along with the ascorbic acid reagent measurement method to determine the accuracy of the speciation experiment. With an average 16% error between the two experiments, it is easy to trust the speciation results. In addition, the standard deviations for loosely-bound phosphorus, iron- and aluminum oxide-bound phosphorus, calcium-bound phosphorus, and residual phosphorus were 3.04, 20.4, 37.7, and 4.5 mg P/kg dry sediment, respectively.

This task also begins to answer the questions and prove the hypotheses put forth at the introduction of this thesis concerning phosphorus sediment flux. It was proposed that phosphorus sediment flux is mostly a function of the physical conditions of the site since the phosphorus cycle is redox dependent. Based on the mostly negative unspiked phosphorus fluxes from Task 1 and the fact that majority of the phosphorus in the sediment is not readily mobilized, it seems that the fate of phosphorus in the wetlands' ambient water is ultimately the sediment itself.

Conclusions for Task 2

Several major implications could be found from the phosphorus speciation study.

- Sediment from each site showed the highest amount of calcium-bound phosphorus.
- No immediate, visual correlation could be found between phosphorus in sediment and phosphorus sediment flux.
- With exceptions, the lowest concentration of phosphorus was attached to the bioavailable, loosely sorbed phosphorus.

Task 3: Determine the Rates of Nitrification and Denitrification Using Serum Bottle Tests

One of the key objectives of this study is to fully understand the nitrogen cycling capability of the bacteria in sediment. In order to explain these processes, multiple nitrogen disappearance rate tests as well as bacterial quantification and identification were carried out.

Bacterial Quantification

Quantification of the nitrifying bacteria using the *amoA* gene in the sediment of each site proved to be a relevant parameter in sediment flux, and the results are shown in Figure 4.5. The overarching trend was that the amount of *amoA*-coded bacteria increased from early summer to late summer sampling, which would be the result of bacterial growth during the three month separation in testing the sediments. Areas such as Unit 1 and South Area displayed dramatic increases in ammonia-oxidizing bacteria populations.

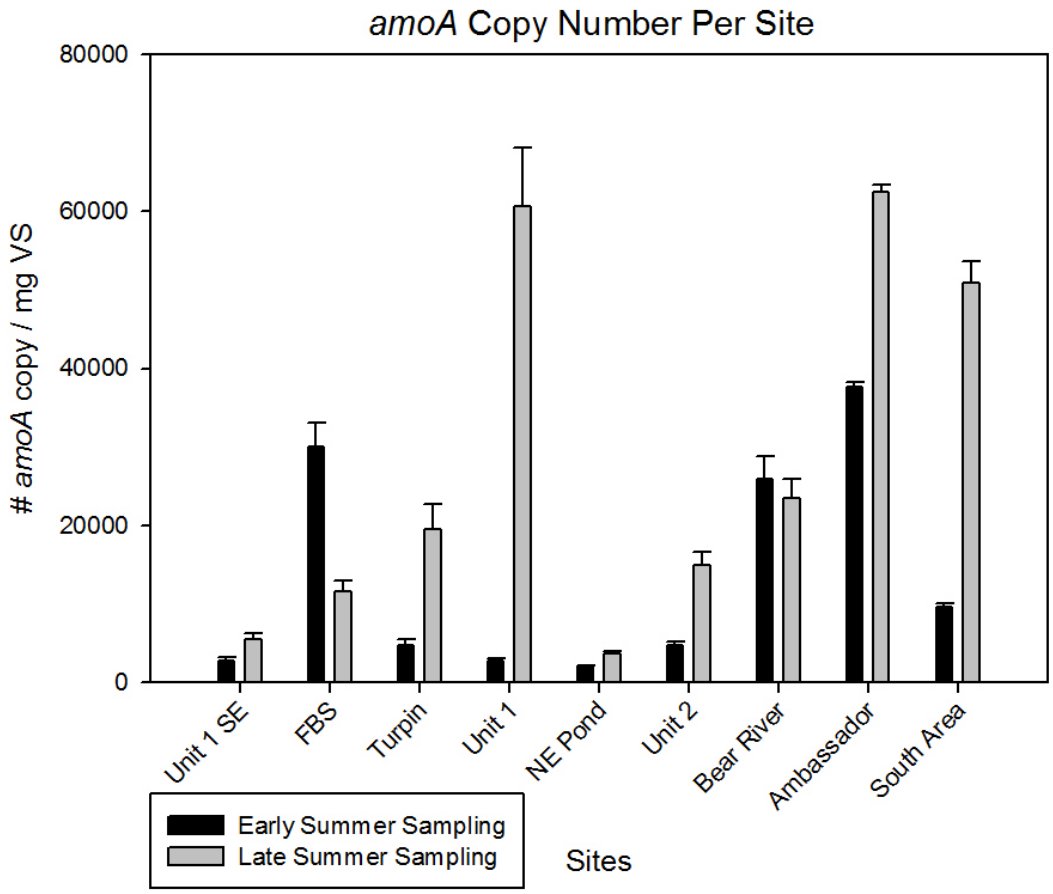

Figure 4.5 *amoA* gene abundance in wetland sites

The reasoning behind Unit 1's increasing *amoA* numbers could lie in the fact that the site was dredged in the winter beforehand, therefore the top layer of sediment was primarily clay. As time passed over the summer, fresh organic mass could have accrued over the clay layer to allow bacterial populations to increase dramatically. All in all, the amount of AOBs present reflects other literature amounts. While this study displayed *amoA* at a range of 3.58 x 10^4 to 1.81 x 10^6 *amoA* copies per gram dry sediment, a different study confirmed their population range as 1.2 x 10^6 to 1.9 x 10^7 copies per gram dry sediment (Sims et al., 2012). Therefore, the wetlands of this study display slightly less *amoA* copies than in other reports, possibly due to the fact that AOB mostly thrive in areas of high ambient ammonia

concentrations (Sims et al., 2012). Perhaps the majority of the wetlands in this study do not display a high enough ammonia concentration to allow AOB to be the dominant ammonia-oxidizing lifeform in the sediments—ammonia-oxidizing archaea tend to thrive in low-concentration environments (Sims et al., 2012). More attention to the ratio of AOA to AOB may be necessary to help further explain the role of microorganisms in nitrification.

Denitrifying bacteria are harder to identify because many heterotrophic organisms carry the same gene for denitrification, *nirS*. As a result of the many possibilities, TRFLP of *nirS* was not carried out during this project, but quantification of the target denitrification gene was focused on instead in Figure 4.6.

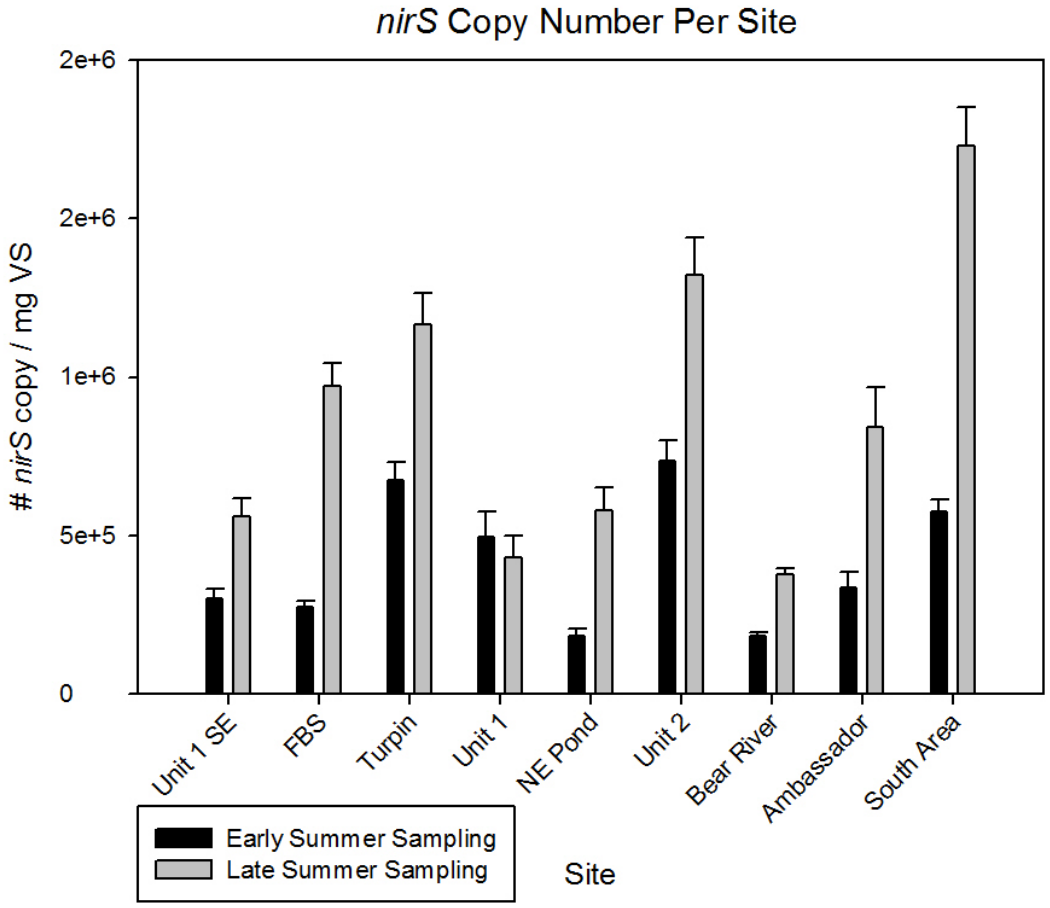

Figure 4.6 *nirS* gene abundance in wetland sites

Changes in denitrifying bacterial populations, represented by quantifying the *nirS* gene, showed similar trends to those for ammonia-oxidizing bacteria. Bacterial populations increased between early summer sampling and late summer sampling, with another great increase in bacterial count for South Area. Unit 1, on the other hand, actually displayed decreased denitrifying bacterial population from May to August despite the huge increase in ammonia oxidizing bacteria during the same period. Nevertheless, other studies have shown riparian wetland denitrifying population numbers to be $1.4 \times 10^7 \pm 2.7 \times 10^6$ copies *nirS* per gram dry sediment (Kim et al., 2008). So the range for this project, $3.44 \times 10^6 - 4.88 \times 10^7$ copies *nirS* per gram dry sediment, is not far off. The growth in bacterial population from early to late summer can be ascribed to increased exposure to higher water temperatures, which should encourage cell growth.

Bacteria Normalized Serum Bottle Rates

To ascertain the ability of each site's sediment to remove nitrogen from the water column, laboratory serum bottle tests were conducted. These included nitrification and denitrification serum bottle experiments to monitor the disappearance of ammonia and nitrate in solution when exposed to the sediment, respectively. As mentioned in the methods section for serum bottle tests, the nitrification serum bottle tests were spiked with and without ammonia as a nitrogen source (0.5 mg/L NH_4-N). Similarly, denitrification serum bottle tests incorporated bottles with nitrate-nitrogen and carbon from acetate, extra nitrogen without extra carbon, and without any carbon or nitrogen spikes (0.5 mg/L NO_3-N, 25 mg/L C from acetate). The serum bottles were shaken at 100 RPM to ensure that transport limitations of nutrients in the serum bottles were negligible. The spiking concentrations were determined to remain consistent with in situ spiking, but there were

differences in relative spiking concentrations between nitrification and denitrification tests. Due to the high concentration of unspiked initial ammonia (sometimes as high as 1.2 mg/L NH_3-N in nitrification serum bottle tests), the ratio between the amounts of ammonia spiked and prespiking ammonia concentration was 0.710 on average. On the other hand, nitrate concentrations in denitrification serum bottle tests were much lower, resulting in the 0.5 mg/L NO3-N spike having a 10.4:1 ratio with initial serum bottle nitrate concentrations. While the difference in spiking ratios could be a reason for more increased denitrification when spiking than nitrification, the spiking ratios showed no correlation for individual site nitrification or denitrification rates. Findings were averaged between duplicates for both the early summer and late summer sampling events, and the results are shown in Figure 4.7 and Figure 4.8.

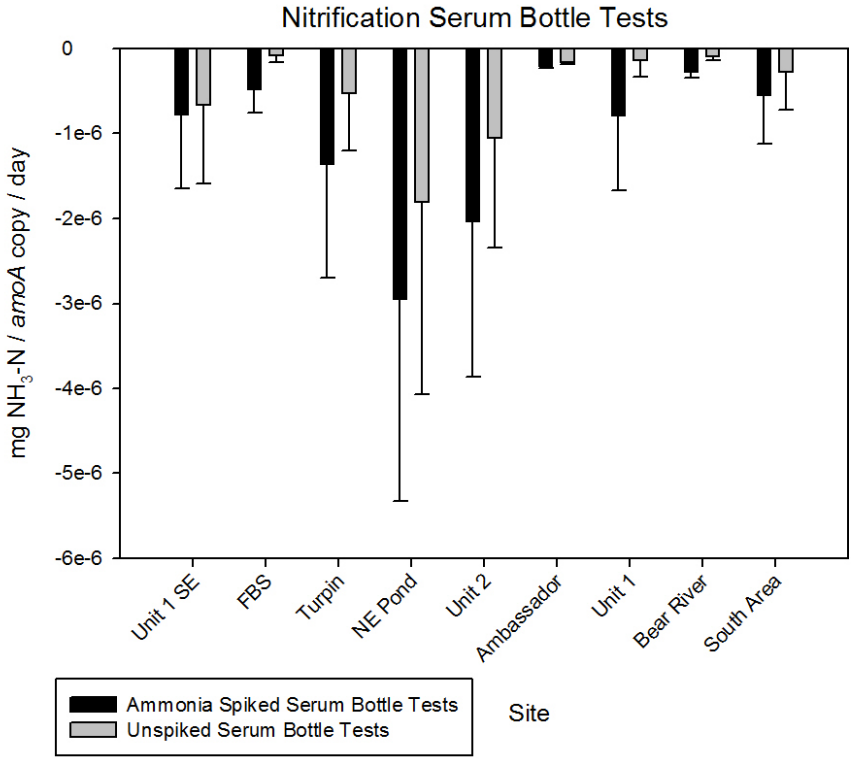

Figure 4.7 Serum nitrification rates normalized to gene copy

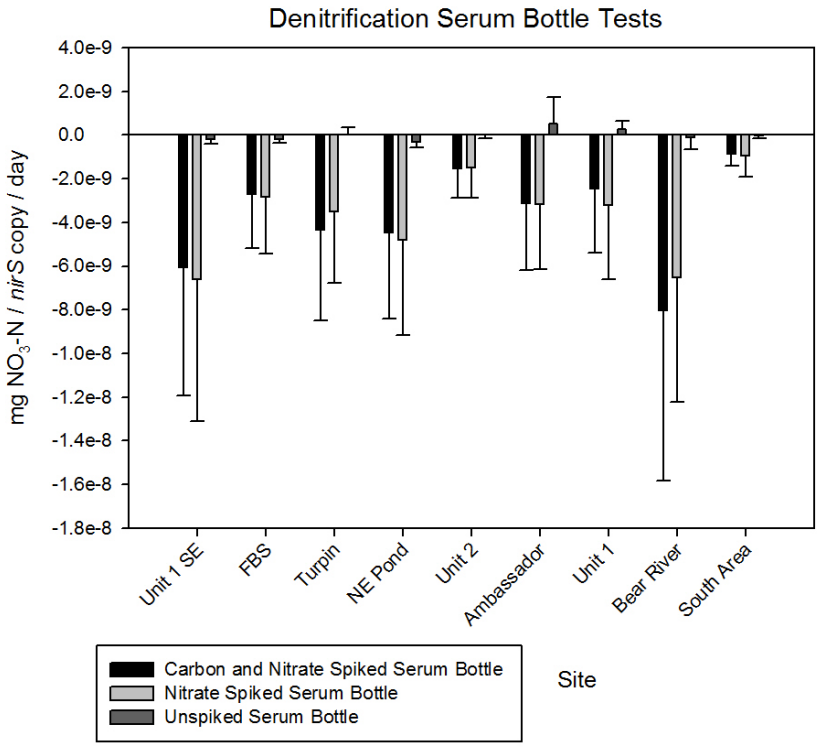

Figure 4.8 Serum denitrification rates normalized to gene copy

The increase in nitrification rates for every site (except SE Unit 1) suggests that bacterial growth in sediments for nitrification is, in fact, nitrogen limited. However, due to the small difference in nitrification between spiked and unspiked serum bottle tests, a paired t-test was conducted to confirm that spiking the serum bottles caused a statistically significant increase in nitrification rate. With a two-tailed P value of 0.0002, the differences between spiked and unspiked nitrification rates were statistically significant. Nitrification is still occurring using ammonia from the pore water, but the nitrification potential is high. NE Pond showed the highest amount of nitrification per target gene, but this could be more of a result of the low *amoA* gene count for the site. Similarly, the denitrification serum bottle tests do not show an unspiked rate for any of the sites because the unspiked denitrification rates were not distinguishable from zero for each site, meaning that the denitrification rate

dramatically increases with incorporation of more nitrate (P value of 0.0001). Meanwhile, very little change in denitrification rates occurred with exposure to carbon spikes. In fact, a paired t-test for carbon and nitrate serum bottle tests versus serum bottle tests only spiked with nitrate showed a two-tailed P value of 0.9497, meaning that the difference between the two sample populations was not statistically significant. The lack of dependency on carbon is in line with the total organic carbon to nitrogen ratios found for sediments in this project's previous study. Usually, the C:N ratio is 20:1 (Craft et al., 2002) while this project demonstrated an average ratio of 12:1.

Due to the high concentration of unspiked initial ammonia (sometimes as high as 1.2 mg/L NH_4-N in nitrification serum bottle tests), the ratio between the amounts of ammonia spiked and prespiking ammonia concentration was 0.710 on average. On the other hand, nitrate concentrations in denitrification serum bottle tests were much lower, resulting in the 0.5 mg/L NO_3-N spike having a 10.4:1 ratio with initial serum bottle nitrate concentrations. While the difference in spiking ratios could be a reason for more increased denitrification when spiking than nitrification, the spiking ratios showed no correlation for individual site nitrification or denitrification rates. Nevertheless, denitrification serum bottle tests showed an average of a 14x rate increase when spiking with nitrate as compared to just a 2x rate increase for nitrification serum bottle tests when spiking with ammonia.

There are multiple ways to analyze the serum bottle tests performed during this project. One way to analyze Figures 4.7 and 4.8 would be to represent the results as an indicator of the performance of individual microbes (for example, one AOB at Turpin is responsible for ~-2.5 x 10^{-7} mg NH_4-N/day in unspiked conditions, only to rise to ~-5.0 x 10^{-7} mg NH_4-N/day for spiked conditions), but this neglects an important point: bacteria are constantly replicating and increasing, especially when in contact with a bioavailable food

source (Metcalf and Eddy, 2003). Unfortunately, qPCR results were not calculated for sediments after a serum bottle test, so the amount of bacteria in each test is unknown. However, the observed changes in nitrification rates versus denitrification rates correspond well with microbiological concepts. Nitrifiers are slow growers compared to denitrifiers due to the extra energy spent by the autotrophic nitrifying bacteria to convert carbon dioxide to pyruvate for metabolism (Metcalf and Eddy, 2003). On the other hand, denitrifying bacteria are heterotrophic, meaning that the bacteria grow faster due to not having to include that extra metabolic step in carbon dioxide conversion (Metcalf and Eddy, 2003). The end result would be a larger increase in the denitrifying population than the nitrifying population when exposed to an extra nitrogen source. Likewise, both the serum bottle tests and the nutrient sediment flux studies showed more increase in nitrogen conversion when spiking nitrate as compared to spiking with ammonia. An increase in both nitrification and denitrification from spiking serum bottle tests with nitrogen, and the ability of sediment to act as a sink for nitrogen when spiked in the wetlands suggest nitrification and denitrification as the dominant nitrogen transformation paths in the sediments.

Nitrification and Denitrification Rates Normalized to Kilogram Sediment

The results from the serum bottle tests were normalized to wet sediments on site because normalizing the nitrification rate by the amount of AOB present may give more information about the number of bacteria in the sediment than the actual nitrification rate. In this way, the results were more comparable to other wetland study results. To make the conversion, the *amoA*-normalized serum bottle rate was multiplied by the amount of bacteria per mass VS and the ratio of VS in the total sediment on site (Figure 4.9).

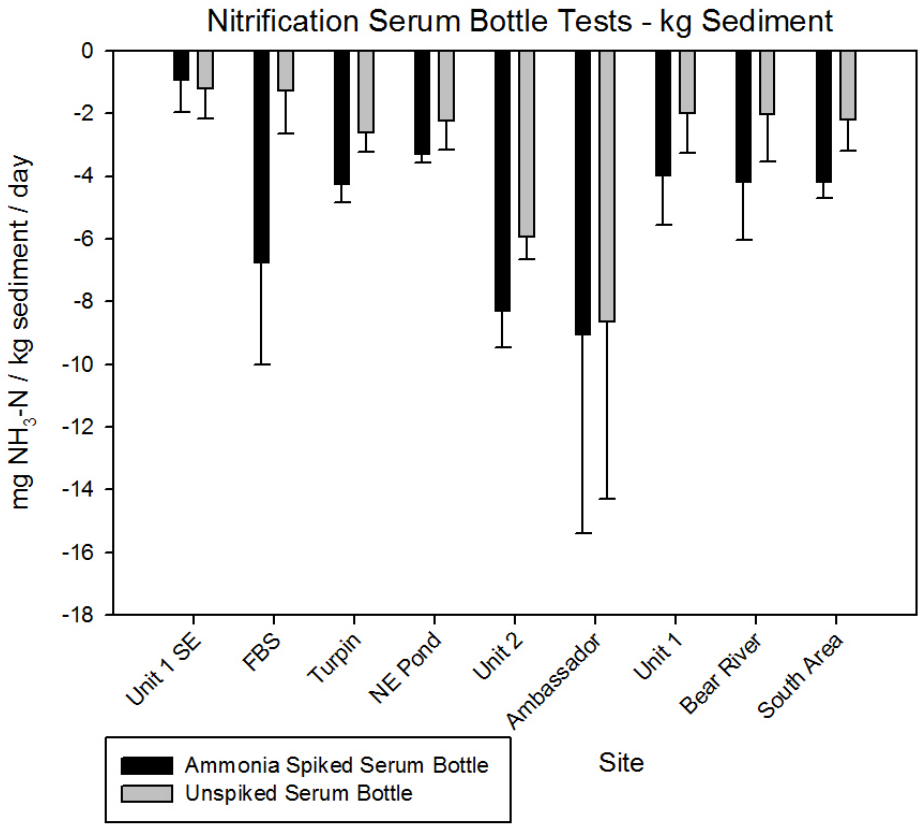

Figure 4.9. Serum nitrification rates normalized to in situ sediment

NE Pond goes from showing the most nitrification per AOB present to having a lower potential nitrification rate. Instead, Ambassador Pond #1 showed the highest amount of nitrification on site, normalized to kg of sediment. SE Unit 1 still shows some of the lowest nitrification rates. A similar study measured nitrification rates in a slurry style as well for prolonged periods of time; however, initial surface sediment nitrification rates of the study showed a mean of 28.5 mg N kg^{-1} d^{-1}, which is similar to the nitrification rates of the spiked serum bottle tests (White et al., 2003). The comparison between the reactors of the White study and the spiked serum bottle test can be maintained because the White study involved constantly aerated sediment, which may stimulate nitrification rates as much as extra bioavailable nitrogen could (White et al., 2003). Unfortunately, nitrification serum

bottle rates were not a good comparison to ammonia sediment flux in the wetlands because, despite Ambassador showing the highest nitrification rate from serum bottles, all sampling events for the site showed the sediment as an ammonia source. Explanations include the bacteria on site possibly not receiving enough oxygen for nitrification or decomposition processes and thus impeding nitrification on site. The denitrification results were considered and approximated as in situ denitrification rates, as well, in Figure 4.10. The denitrification rates from serum bottle tests reflected more what was observed with sediment nitrate fluxes than nitrification serum bottle tests as, like the spiked nitrate sediment fluxes, spiked denitrification serum bottle tests all fell within roughly the same range—they displayed fewer fluctuations than the nitrification serum bottle tests.

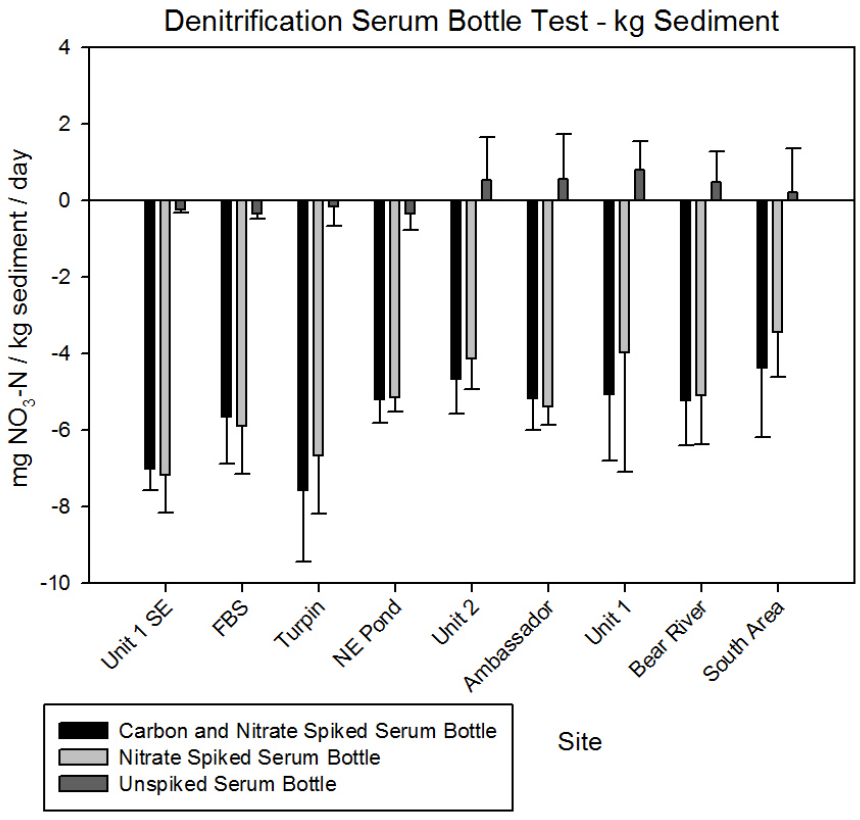

Figure 4.10 Serum denitrification rates normalized to in situ sediment

While the denitrification rates may seem low, it should be noted that the nitrification serum bottle tests likely received more oxygen than the sediments in wetland settings, thus stimulating nitrification more so than denitrification in the laboratory setting. The comparison study (White et al., 2003) showed lower rates for denitrification than nitrification as well. In the study, the sediment from the surface, analyzed in anoxic conditions, showed a denitrification rate of 13.1 mg N kg^{-1} day^{-1} (White et al., 2003). This is higher than what was observed even for the spiked denitrification serum bottle tests, though the observed rate in White was from sediment extracted from their nitrification reactor; it's likely their denitrification rate was determined from water with elevated nitrate concentrations as well (White et al., 2003). Also, the White value for denitrification is a potential value, meaning the highest rate of denitrification observed during a period of the study was reported (White et al., 2003). The serum bottle test results were then used to create a nitrogen mass balance for the sediment.

Serum Bottle Nitrogen Mass Balance

One last advantage to serum bottle tests includes the ability to survey the pathways of nitrogen transformations in the sediment slurry. The initial assumption for mass balance in this section was that all ammonia was being converted to nitrate and nitrite in equivalent, stoichiometric terms, as nitrification is substrate-limited (the substrate in this case being ammonia). However, it soon became obvious that simultaneous nitrification and denitrification were occurring in the nitrification serum bottles, as the dissolved oxygen concentration must have gotten low enough to create microanoxic zones in the sediments, allowing for simultaneous nitrification and denitrification.

For example, during an early summer nitrification serum bottle test for NE Pond,

the initial ammonia concentration measured was 0.486 mg/L NH_3-N, with a final concentration of 0.191 mg/L NH_3-N. If denitrification were not occurring, one would expect the NO_2-N and/or the NO_3-N concentration to increase by 0.295 mg/L NH_3-N, barring any microbial nitrogen uptake. Instead, the NO_2-N concentration increased from 0.004 to 0.012 mg/L NO_2-N and the NO_3-N concentration actually decreased from 0.54 to 0.019 mg/L NO_3-N. These same kind of serum bottle results were shown in almost all of the nitrification tests, indicating that denitrification must have been occurring as well (for this reason, nitrification was measured using ammonia disappearance).

Instead, serum bottle tests were used to quantify a rate of nitrogen loss in the system via liberation of nitrogen gas after denitrification. In order to perform this mass balance, though, a rudimentary determination of decomposition rates had to be inferred from the results. Both decomposition and DNRA primarily occur in anoxic conditions and are the leading source of ammonia in sediment, therefore it was assumed that these processes would be the most likely source of ammonia during the denitrification serum bottle tests (DeBusk et al., 2001). During the May serum bottle tests, ammonia concentrations were recorded at the beginning and end of unspiked denitrification reactions to formulate an assumption for the anoxic rate of ammonia creation for each site, and the results are shown in Table 4.10. The May sampling event was picked to measure anoxic ammonia changes as there was assumed to be more organic matter undergoing decomposition, since during the winter and springtime, decomposition may occur slowly. As a result, decomposition processes were assumed to be higher in the early Summer event than late Summer. Interestingly, FBS showed the highest hourly anoxic ammonia increase rate, while sites such as Bear River Unit 5C, Ambassador Pond #1, and SE Unit 1 showed the lowest hourly anoxic ammonia increase rates.

Table 4.10 Anoxic Increase in Serum Bottle Tests

Hourly Anoxic NH_4-N Increase (ppm/hr)	
Site	May
SE Unit 1	0.0028
FBS	0.0829
Turpin	0.0231
NE Pond	0.0143
Unit 2	0.0450
Ambassador	0.0025
Unit 1	0.0292
Bear River	0.000
South Area	0.0193

These rates were accounted for when creating a nitrification nitrogen balance amount for each site during both the early summer and late summer sampling. The nitrogen budget was created by measuring the change in TIN in the unspiked nitrification serum bottle tests. Initially, an overall increase in TIN was observed, but after subtracting out the anoxic ammonia increase rates (from ammonification and DNRA), a negative mass balance was displayed for all sites (Figure 4.11). The negative nitrogen mass balance proved that in these isolated laboratory conditions, the sediment was overall removing nitrogen – in situ results may vary. After all, laboratory serum bottle tests involved agitation of the sample containers, which stimulates bacterial activity and does not occur naturally.

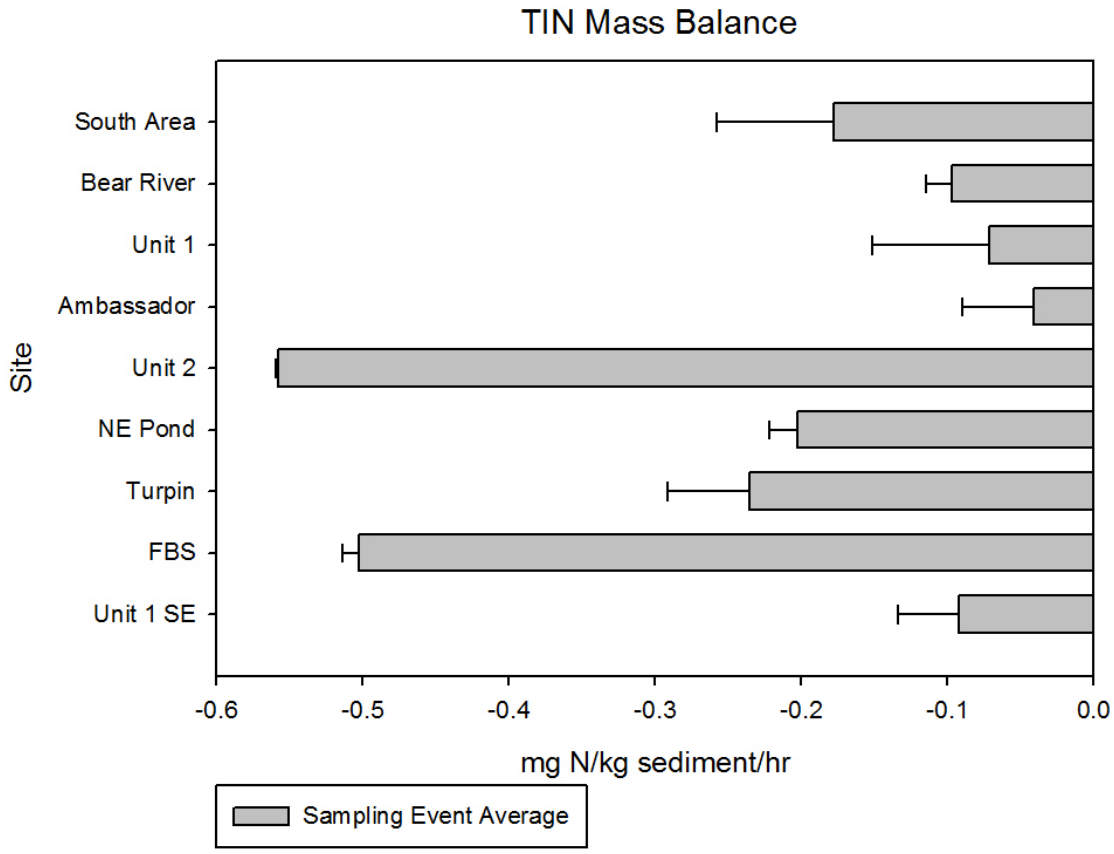

Figure 4.11 Site specific serum bottle mass balance

Overall, each site showed a decrease in the inorganic nitrogen pools of the serum bottles. This is to be expected as denitrification was occurring. One of the biggest takeaways from the nitrogen balance is which sediments show the most potential in nitrogen removal. The Unit 2 and FBS sites overshadow the others, as more than a part per million of nitrogen each escaped as nitrogen gas during the course of the experiment. Unit 1 and Ambassador showed the lowest overall nitrogen removal rates, and, interestingly, sites with the highest concentration of ambient TIN displayed the most nitrogen removal in the sediment. This suggests that TIN removal for the sediments follows a first-order rate at these concentrations.

The important message from the nitrogen mass balance results of the serum bottle tests show that overall, the majority of the nitrogen removal occurs in the sediments. This shows promising results that sediment at the sites can be used to remove nitrogen contaminants, most likely through combined nitrification denitrification. As for the enhancing of nitrification and denitrification rates, additional carbon may not be necessary at the wetland sites at current nitrogen loading rates.

Conclusions for Task 3

A series of serum bottle tests weas able to provide conclusions based on metabolic availability and nitrogen mass balance as shown below.

- In laboratory conditions, all sediments proved to be overall nitrogen sinks.
- Each site showed a nitrogen dependency for nitrification and denitrification, as rates increased after adding ammonia or nitrate. Rates for denitrification were unchanged with added bioavailable carbon.
- Nitrification occurred in the sediments at a faster rate than denitrification, and nitrification showed less nitrogen dependency than denitrification.

Task 4: Identify Bacteria Participating in Nitrification and Denitrification Using Advanced Molecular Tools

While the physical properties of the soil and ambient water column are important, one of the goals of this report was to measure the significance of the bacteria to sediment nutrient interactions in the wetland. Also, the species of bacteria on site may be important if a pattern between species of bacteria present and nitrogen cycling rates can be found. A closer observation of the relationship between bacterial population size and nitrification and

denitrification rates shows that quantification alone cannot explain the full picture of bacteria interactions in the sediment. Figures 4.12 and 4.13 represent this relationship. A small R^2 value was depicted to show that there was, in fact, no linear correlation between bacterial gene copy and nitrification and denitrification rate. The number of gene copies was quantified to represent the bacterial population size in each individual serum bottle. As shown, no rate showed sufficient correlation to the amount of bacteria in the sample (though spiked nitrification rates showed the strongest correlation at $R^2 = 0.237$).

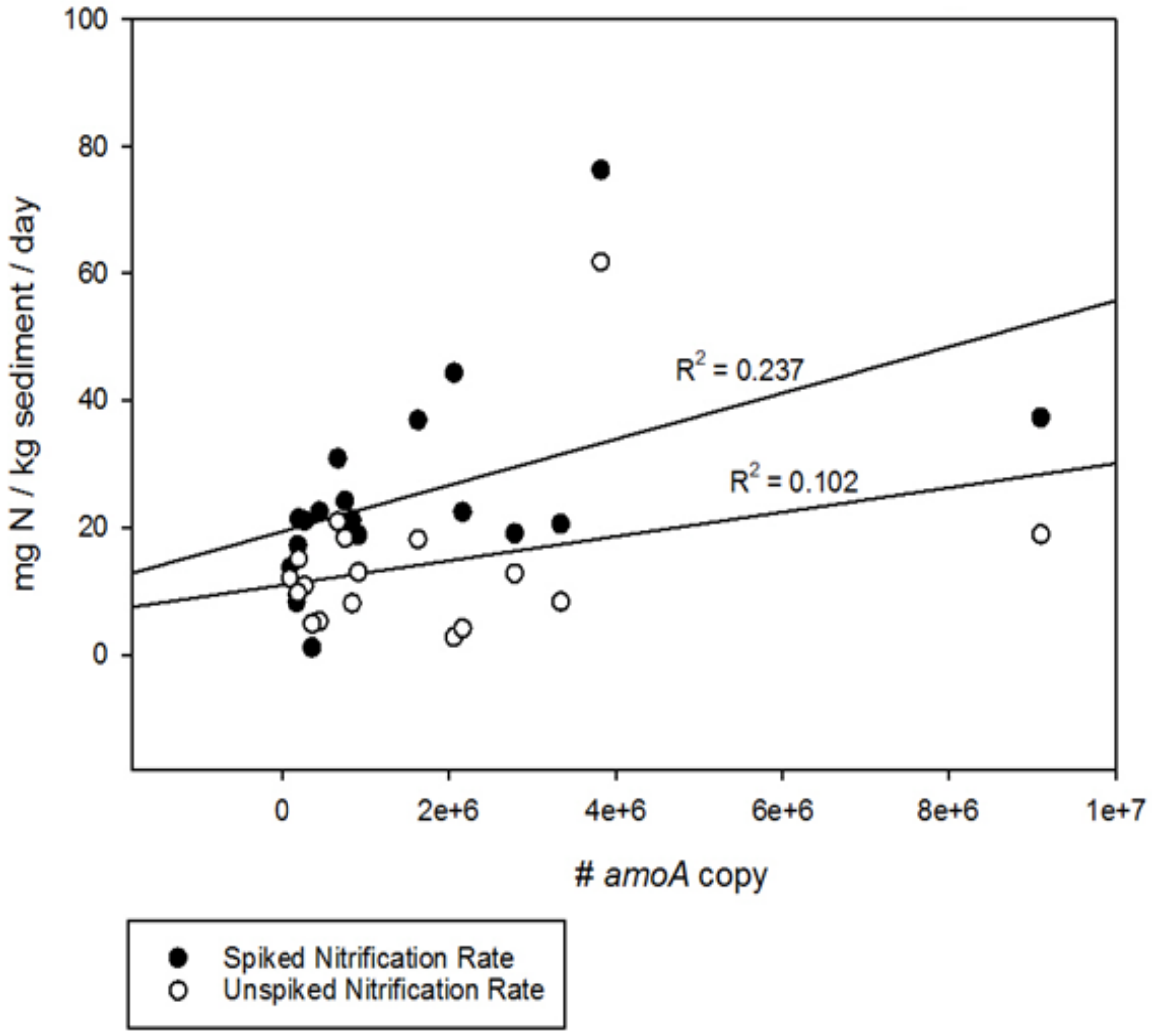

Figure 4.12 Nitrification and bacterial population size in serum bottles

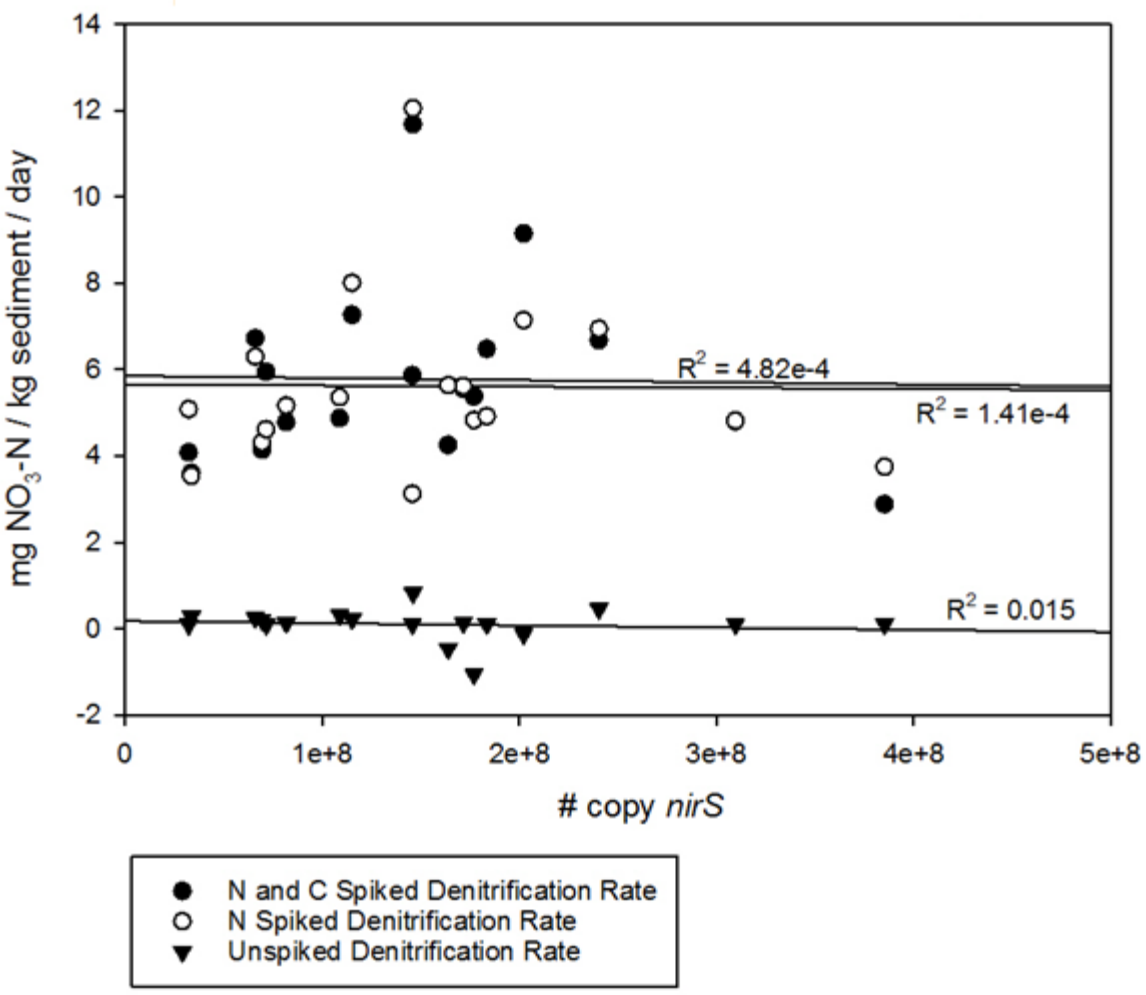

Figure 4.13 Denitrification and bacterial population size in serum bottles

Therefore, many other factors must be at play, including the species of nitrifiers and denitrifiers present. Other studies have shown that each AOB species nitrifies at a different rate, and the lack of correlation between bacterial population count and nitrogen cycling rate support this observation (Dytczak et al., 2008).

Genomic Analyses

The results of the T-RFLP analysis are displayed in Figure 4.14. This figure was used to further identify nitrifying species of bacteria.

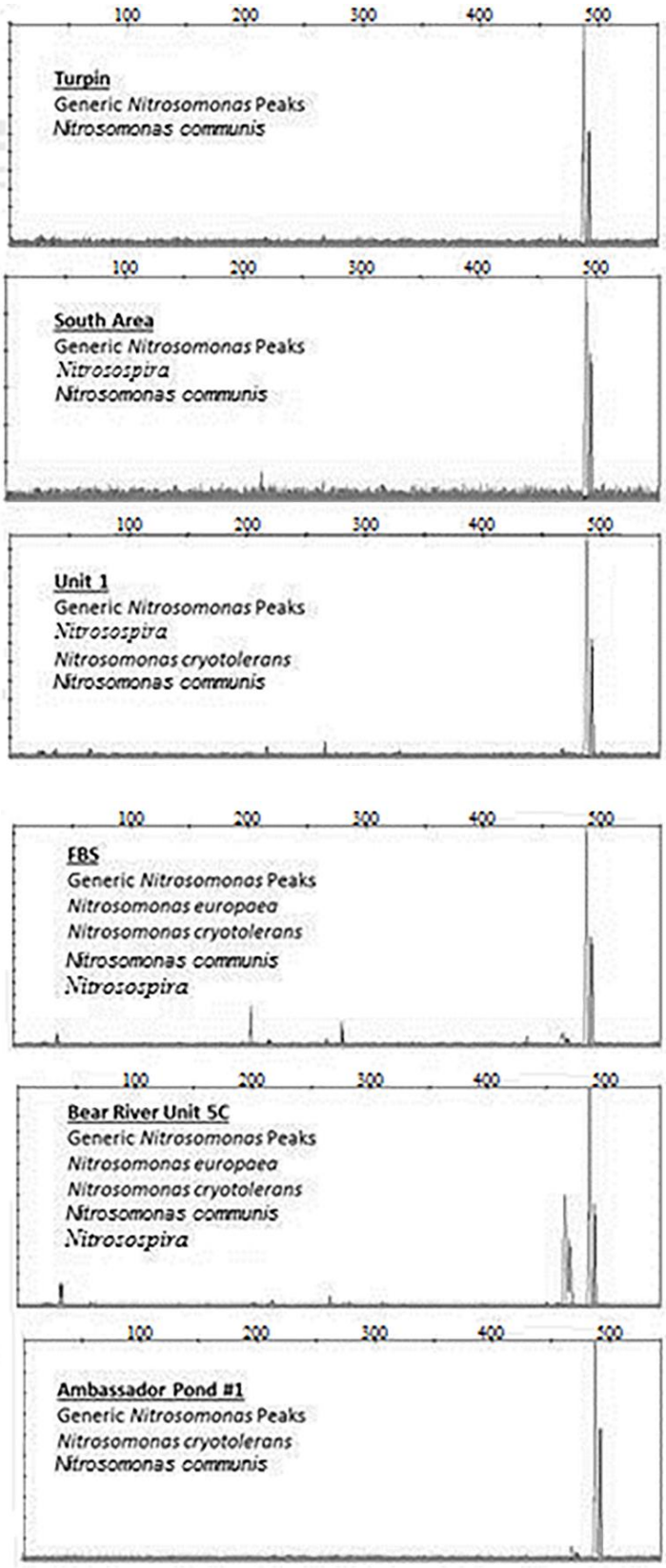

Figure 4.14 TRFLP results for the AOB community

Figure 4.14 continued

The peaks in the above eletrograph give evidence for the types of *Nitrosomonas* species present in addition to presence of the genus *Nitrosospira*. Interpretation of terminal restriction fragment length polymorphism electrographs includes matching the forward (green) and reverse (blue) peaks to the AOB species in the following Table 4.11 (the peaks are identified as terminal fragment size). Most species show multiple peak combinations in the resulting electrographs. TRFLP results were followed by DNA sequencing to produce phylogenetic trees.

The most prevalent species of AOB identified with TRFLP for all sites was *Nitrosomonas communis*, which tend to proliferate in mid-range ammonia concentration conditions with low salinity (Koops et al., 1991). This species was identified with the 491/491 peak, although the 491/491 peak is the generic peak for all *Nitrosomonas* species (Siripong et al., 2007). Further sequencing investigation would be needed to confirm the assumption that the 491/491 peaks are for *N. communis*. *Nitrosomonas oligotropha* was not identified for any of the sights – the absence of this bacteria could be used as in indicator for wetland contamination in the future. The presence of *Nitrosomonas europaea* could prove the alternative – that enough nitrogen contamination exists to support this bacteria.

Table 4.11. AOB species and corresponding TRFLP electrograph peaks

Nitrifier Group	TF size (bp) Forward/backward
Nitrosomonas europaea	219/270, 491/491
Nitrosomonas oligotropha	48/135, 354/135, 491/491
Nitrosomonas cryotolerans	48/441, 354/48, 441/48, 491/491
Nitrosomonas marina	48/441, 48/135, 491/491
Nitrosomonas communis	491/491
Nitrosospira	283/206

Another prevalent species of AOB identified with TRFLP at the sites is *Nitrosomonas cryotolerans*, which requires salinity to survive and can endure temperatures of below 0° C (Koops et al., 1991). This corresponds well with the yearly climate of Salt Lake City, Utah. In addition, FBS and Bear River Unit 5C showed the presence of *Nitrosomonas europaea*; for FBS, the site's close proximity to the South Davis North's effluent may cause more of these bacteria to be present as *N. europaea* thrive in WWTPs (Koops et al., 1991). Last, the genus *Nitrosopira* appeared in a few sites – this is to be expected as *Nitrosospira* is one of the two β subclasses of *Proteobacteria* which conduct ammonia oxidation, along with *Nitrosomonas* (Schramm et al., 1998).

To prove the existence of the identified bacterial species from TRFLP, cloning and sequencing was carried out. In this case, the top three sites with the highest ammonia disappearance rates were chosen for identifying nitrification bacteria. Unit 2, Unit 1, and South Area were chosen to sequence nitrifying bacteria. Hopefully these sites would create a representative sample for the entire project – the results are shown in Figure 4.15. Results from the *amoA* tree were conclusive – the 491/491 TRFLP peaks were, in fact, evidence of *N. communis*. In addition, sequencing showed evidence of *N. ureae* being present in the soil. This bacteria uses urine as a nitrogen source (Koops et al, 1991). The phylogenetic tree of the *nirS* gene-carrying denitrification community (Figure 4.16) did not show as conclusive results as the *amoA* phylogenetic tree. The sites that were the three largest sinks of nitrate during the sediment nutrient flux study were chosen for sequencing – NE Pond, Unit 2, and SE Unit 1. The results were in line with the evidence that the denitrification community is very diverse (Kadlec et. al, 2009). To properly identify all of the denitrification bacteria present, it is speculated that multiple gene indicators must be used, as no single identification gene is ubiquitous among all denitrification bacteria.

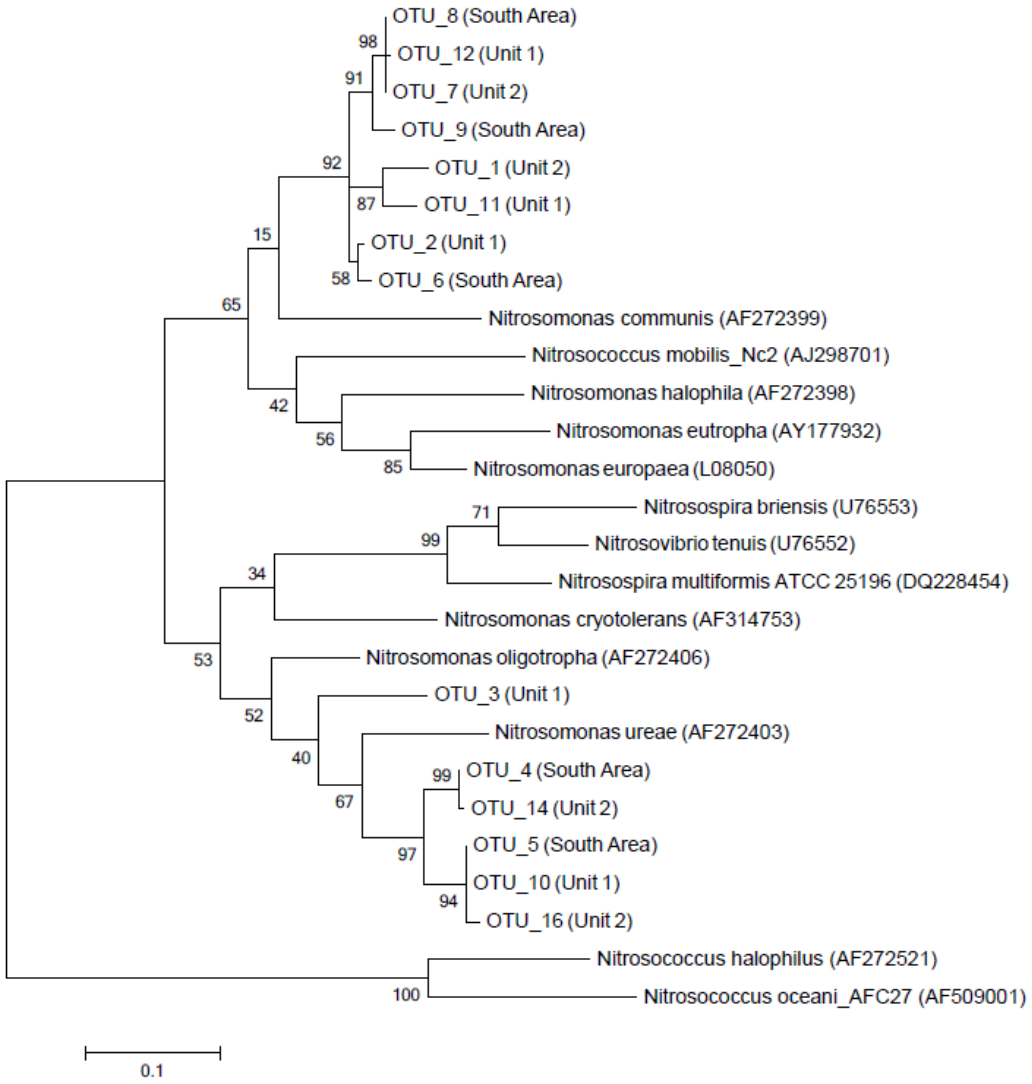

Figure 4.15 *amoA* phylogenetic tree

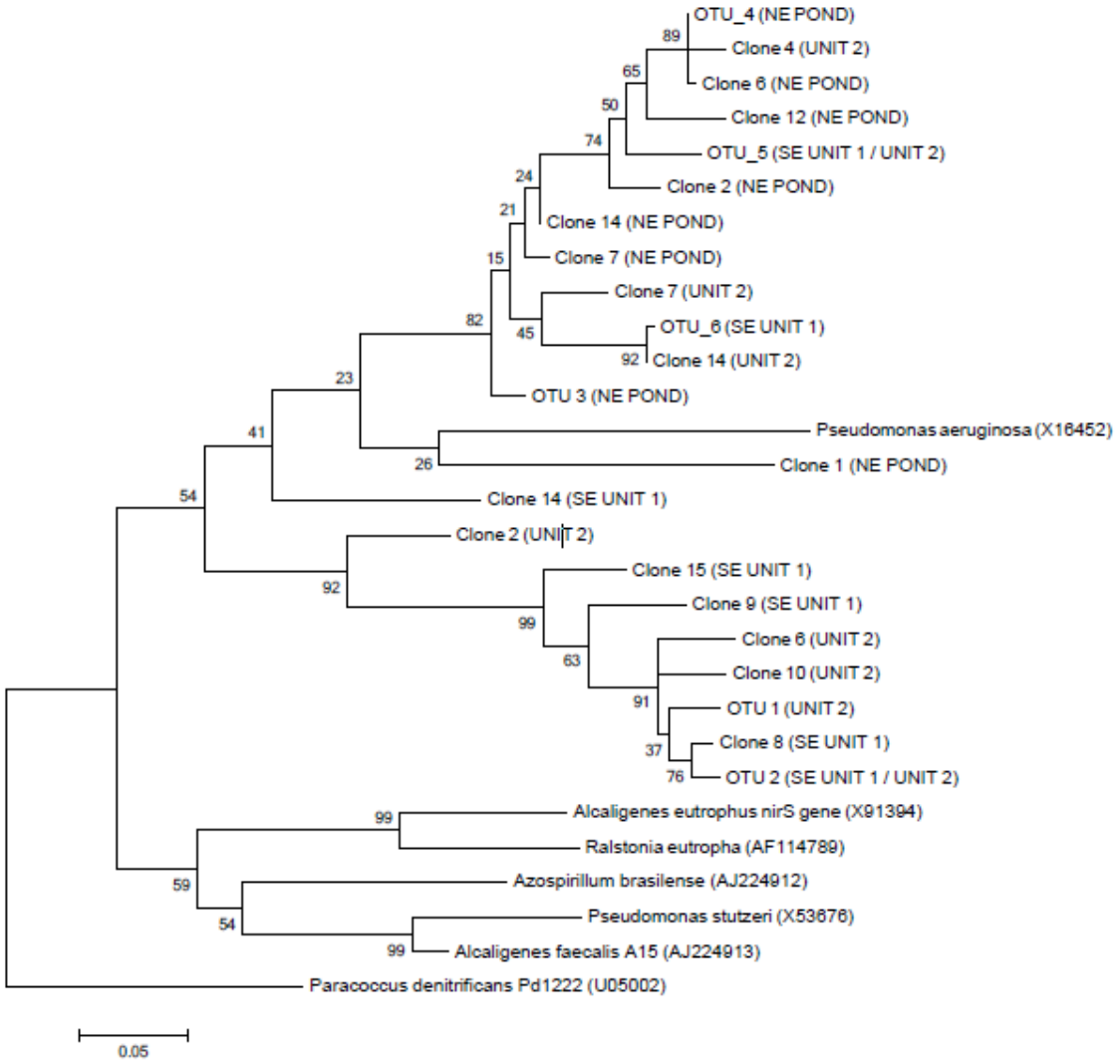

Figure 4.16 *nirS* phylogenetic tree

The sites that were the three largest sinks of nitrate during the sediment nutrient flux study were chosen for sequencing – NE Pond, Unit 2, and SE Unit 1. The results were in line with the evidence that the denitrification community is very diverse (Kadlec et. al, 2009). More genera were considered than the nitrification community sequencing analysis, and fewer species showed resemblance to the identified OTUs than with the nitrification sequencing analysis. In addition, more OTUs were identified during sequencing analysis than with the nitrification community. The closest species to an OTU identified with the *nirS* phylogenetic analysis was *Pseudomonas aeruginosa*. This species of bacteria can be found in soils, marches, and marine habitats, as well as on plant and animal tissues – it is truly a ubiquitous species (Stover et al., 2000). *P. aeruginosa* also is one of the top causes of opportunistic human infections as the bacterium is inherently resistant to antibiotics and disinfectants (Stover et al., 2000). Other than this species, no other *nirS* containing denitrifying species with sequences analyzed in this project showed a close relation to the OTUs. Due to the variety of bacteria encountered in wetland sediments, and in the natural environment as a whole, many OTUs would be expected. In addition, the denitrification community is studied less than the nitrification community, so less academic knowledge of the exact denitrification community structures is to be expected. Had this study sequenced bacteria from a bacterial isolate culture, the phylogenetic tree would likely have been more exact – Figure 4.16 is an example of how much variety exists for a single bacteria community in the environment.

Conclusions for Task 4

Many steps were taken to draw conclusions about the bacterial community of the wetlands. The focus genes for sequencing was *amoA* for nitrifying species and *nirS* for

denitrification species. The following conclusions were made:

- Bacterial community composition could be as important as community size with regards to nitrification and denitrification rate.
- The most common AOB in the wetlands was *Nitrosomonas communis*, as identified with both TRFLP and phylogenetic tree analysis
- No consensus could be made for the exact composition of the denitrification community, partly because of the complexity of the community present.

Task 5: Data Analysis Using Statistical Software "R"

Both the hypothesis that bacterial processes control nitrogen sediment fluxes and the hypothesis that abiotic factors regulate phosphorus sediment flux rely on statistical comparison of the measured variables to the sediment flux values measured on site. After comparison of correlated variables to sediment fluxes, assessments were made on which variables are the most important to sediment flux.

Nitrogen Data Diagnostics

Before principal component analyses were carried out, the sites were tested against each other using a Z-test analysis to observe how each site related to the sample mean. If any sites varied far enough from the sample mean to become multivariate outliers, this may have altered the PCA results. The preliminary Z-test diagnostics showed Unit 2 as displaying ammonia and nitrate sediment fluxes at least two standard deviations from the sample mean in all observations. A high porosity of sediment at this site was concluded as the reason the nutrient fluxes were so far outside the sample mean for Unit 2. The higher porosity of the sediment could have caused greater mixing of the typically horizontally stacked

biogeochemical reaction zones in the sediments, thus allowing for more bioavailable carbon and nitrogen sources to be available to the nitrification and denitrification bacteria in each zone (Huettel et al., 1998). For example, denitrification could be carried out with more ease deeper in the sediment as the higher porosity allows more organic carbon flux deeper into the sediment. As for nitrification, ammonia created from decomposition deeper in the sediment could reach the sediment surface faster with the higher porosity. Thus, the high porosity of the sediment may be the reason Unit 2 displayed such high sediment nutrient flux values. As a result, Unit 2 was not included in the determination of which variables control nutrient fluxes.

Ammonia Principal Component Analysis

The basis for principal component analyses (PCA) was described in the methodology section of this report. Principal components are linear combinations of multiple variables; two principal components are created during a PCA, which show the least correlation. A principal component can be calculated using the following equation, for example, of which the coefficients for each variable are created through statistical software.

$$PC1 = -0.846*(PO_4\text{-P Flux}) + 0.213*(TOC) - 1.67*(Depth)\ldots.. \quad [25]$$

$$PC2 = 0.254*(PO_4\text{-P Flux}) - 3.73*(TOC) - 0.557*(Depth)\ldots. \quad [26]$$

Biplots are crated using these principal components as the XY-axis of which to plot both the observations (sampling sites) and the variables. Information on both the individual sites of this analysis and the variables measured can be collected by visual interpretations of

PCA biplots. First, observations can be related to one another by observing the "scores" of a PCA. A score is a point on the biplot that is a (x,y) coordinate result of factoring in each observation's many variables into the two principal components which comprise the XY-axis. The following variables were used to create the principal components for the ammonia sediment flux biplot. Bolded variables were assessed for PCA loadings, as will be explained. The variables from Table 4.12 at all sites were used to create a PCA biplot to observe the relationship between each site's scores. This resulting biplot (with variables removed) is shown in Figure 4.17. Each site had four observations; these included both spiked and unspiked sediment ammonia fluxes for both early and late summer sampling. Ideally, each site would show close locations for all four observations, but, as with Ambassador Pond #1, this is not always the case. In addition, sites with proximal groupings behaved more similarly during this PCA, indicating the NE Pond and Unit 2 behaved similarly with regards to ammonia sediment flux for example.

Table 4.12. Ammonia sediment flux PCA variables

Water Physical Characteristics	Ambient Chemical Concentrations	Soil Composition	Serum Bottle Results
Min DO	**NH_3 Conc.**	TOC	**Spiked Serum Bottle Rates**
Max DO	**Salinity Conc.**	**Pore NH_3 Conc.**	Unspiked Serum Bottle Rates
Δ DO	Average npDOC Conc.	Pore SO_4 Conc.	Nitrogen Mass Balance
Min DO Sat %	**NO_2 Conc.**	mg VS Present	1st Order Rate Coefficient
Max DO Sat %	**Chlorophyll-a Conc.**	Dry Density	
Δ DO Sat %	**Turbidity**	Porosity	
Min pH		**# of *amoA* copies present**	
Max pH		**NH_3 Conc. Gradient**	
Δ pH			
Min Temp			
Max Temp			
Δ Temp			
Depth			

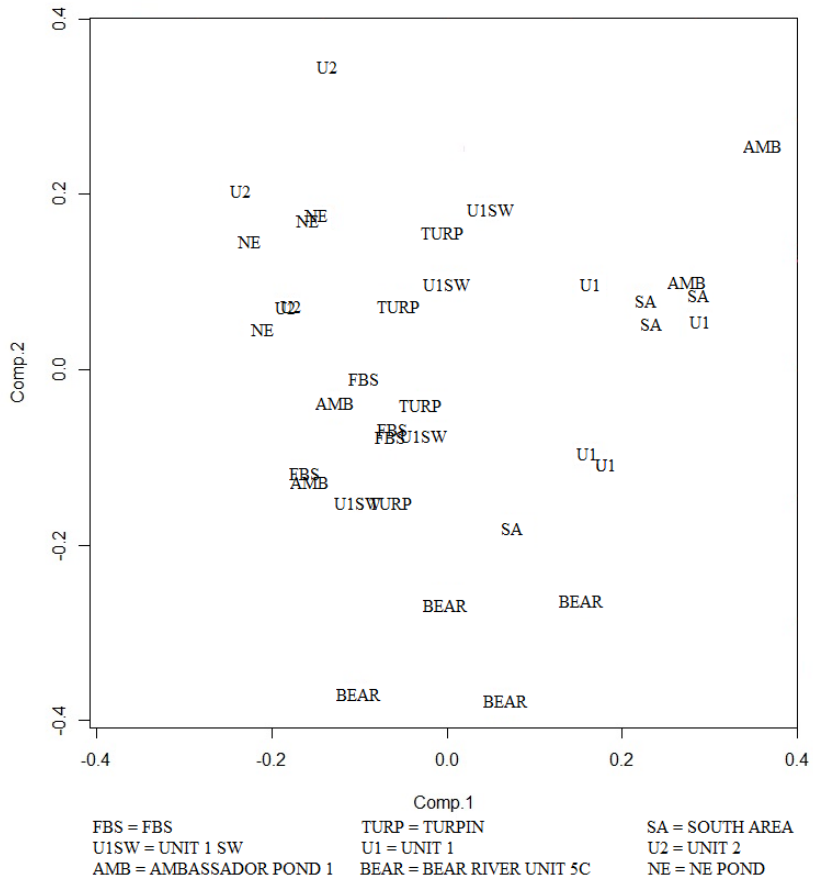

Figure 4.17. Ammonia sediment flux principal component analysis – site scores

Each site had four observations; these included both spiked and unspiked sediment ammonia fluxes for both early and late summer sampling. Ideally, each site would show close locations for all four observations, but, as with Ambassador Pond #1, this is not always the case. In addition, sites with proximal groupings behaved more similarly during this PCA, indicating the NE Pond and Unit 2 behaved similarly with regards to ammonia sediment flux, for example. However, despite their similarities on the PCA biplot, these sites did not show very similar ammonia sediment flux results (Table 4.13). Many factors play in to the differences in biplots.

Table 4.13. Comparing Unit 2 and NE Pond ammonia sediment flux

NH_3-N Sediment Flux (g NH_3-N/m^2/day)		
	May Sampling	August Sampling
Unit 2 Unspiked	-0.563	0.254
Unit 2 Spiked	-0.593	0.053
NE Pond Unspiked	0.000	0.044
NE Pond Spiked	-0.213	-0.025

Therefore, at initial inspection, little can be deduced by the score of each site in the PCA biplots as the number of variables considered for each principal component is so high. Important variables such as ammonia sediment flux are thus outweighed by the multiple other variables also considered. Trimming principal components to only include variables of interest could create a PCA biplot with more straightforward implications. On the other hand, using PCA biplots to interpret "loadings" of variables proved to be more useful than viewing scores.

A variable's loading in PCA is a measure of the coefficients which are multiplied with the variable to create the respective principal component. For example, in Equations 25 and 26, the loading for PO_4-P Flux would be (-0.846, 0.254) for PC1 and PC2 – each loading creates a vector for each variable which originates at (0, 0). As a result, multiple variable-vectors can be plotted at once and visually compared; vectors which are the closest to each other represent correlated variables. The ammonia sediment flux PCA biplot with variables included is shown below; all sites were used for the creation of this graph as well as all variables in Table 4.11. Figure 4.18 displays this resulting biplot – the ammonia sediment flux loading shows some correlation to the ambient ammonia concentration and amount of *amoA* genes present.

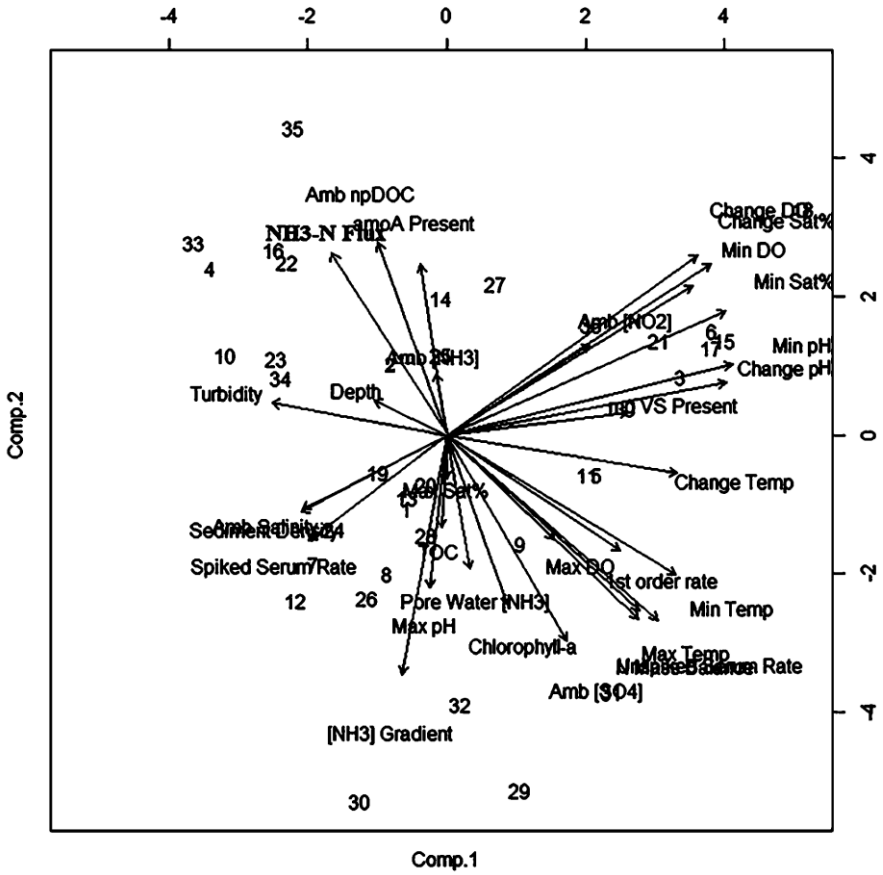

Figure 4.18. Ammonia sediment flux loading biplot - all variables

A biplot with loadings included can be used to interpret which variables are correlated – in this case, the ammonia sediment flux variable is bolded and the amount of *amoA* in the sediment as well as ambient ammonia concentrations, depth of water, and nonpurgable dissolved organic carbon concentration of ambient water all suggested some correlation to ammonia sediment flux. However, this biplot could be inaccurate, as Unit 2 has already been proven to show flux far enough outside the sample mean to suggest the site should not be related to others. Therefore, Unit 2 was removed from the PCA so this outlier would not alter the analysis for all sites. In addition, the number of variables was reduced to remove apparently redundant variables. Proportions of variance explain how

much variance in the observations can be accounted with each principal component – a higher value indicates that the principal component accounts for more variation in the data than lower values (Gabriel, 1980; Montgomery, 2011). The next PCA biplot for ammonia sediment flux, Figure 4.19, only considers the bolded variables in Table 4.11 and neglects Unit 2. These variables were chosen because they showed the most correlation with ammonia sediment flux after removing Unit 2.

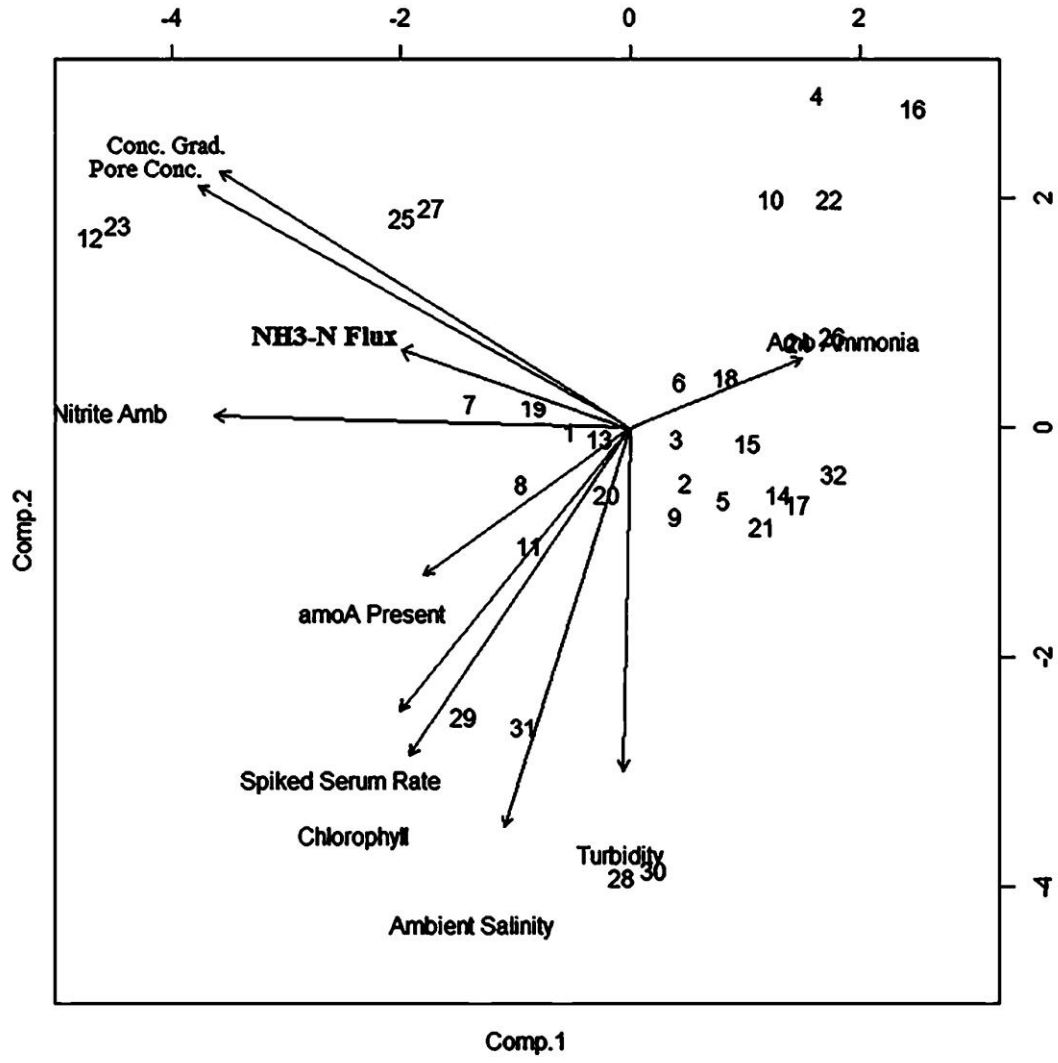

Figure 4.19. Ammonia sediment flux biplot without Unit 2 – select variables

By removing variables, the proportion of variance changed from 0.257 and 0.164 with all variables to 0.263 and 0.199 after variable reduction for PC1 and PC2, respectively. The increasing proportion of variance showed that removing Unit 2 for the analysis helps the principal components explain more of the variation in data; another way to view the variance of principal components is through scree plots. Scree plots show how much variance is accounted for by each principal component eigenvector (Jackson, 1993). A favorable scree plot should show the first several principal components having a much higher variance than the rest of the principal component, causing a peak in points in the first several principal components, followed by a drop-off in variance for the remainder of the principal components (Jackson, 1993). This trend should be evident for all structured, nonrandom data sets (Jackson, 1993). A comparison of the scree plots before and after removing Unit 2 from the ammonia sediment flux analysis is shown below. The curvature of the scree plot suggests that the first several principal components do not represent the data set well (Jackson, 1993). The scree plot for the analysis before removing Unit 2 is shown below in Figure 4.20. Figure 4.21 shows better distribution of variance for removing Unit 2 and using select variables. On the other hand, after removing Unit 2 from the ammonia PCA (Figure 4.19), the result reflected less correlation of ammonia sediment flux with the amount of *amoA* present. This could suggest that more research should be made to quantify the role of archaea in the nitrification cycle in the wetlands. Next, a similar path was followed to explain nitrate sediment flux.

Nitrate Principal Component Analysis

The following variables in Table 4.14 were considered for the creation of the nitrate sediment flux PCA biplot.

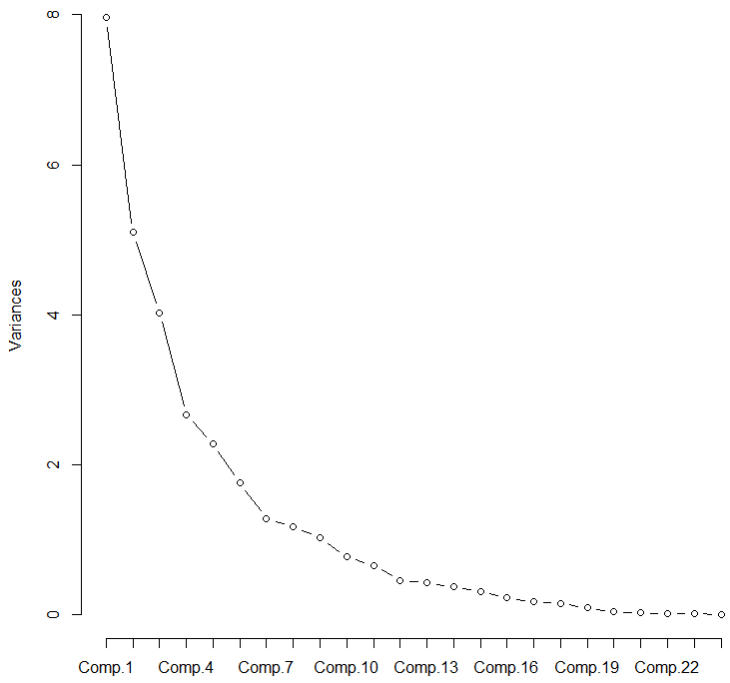

Figure 4.20. Ammonia sediment flux scree plot - all variables

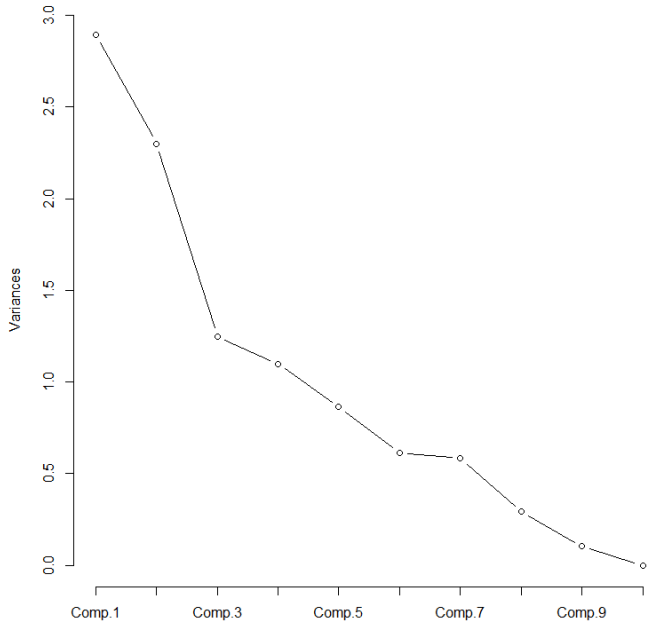

Figure 4.21. Ammonia sediment flux scree plot without Unit 2 – select variables

Table 4.14. Nitrate sediment flux PCA variables

Water Physical Characteristics	Ambient Chemical Concentrations	Soil Composition	Serum Bottle Results
Min DO	NO_3 Conc.	**TOC**	**Spiked Serum Bottle Rates**
Max DO	**Salinity Conc.**	Pore NO_3 Conc.	Unspiked Serum Bottle Rates
Δ DO	**Average npDOC Conc.**	Pore SO_4 Conc.	Nitrogen Mass Balance
Min DO Sat %	NO_2 Conc.	mg VS Present	1^{st} Order Rate Coefficient
Max DO Sat %	**Chlorophyll-a Conc.**	**Dry Sediment Density**	
Δ DO Sat %	**Turbidity**	Porosity	
Min pH		**# of *nirS* copies present**	
Max pH		**NO_3 Conc. Gradient**	
Δ pH			
Min Temp			
Max Temp			
Δ Temp			
Depth			

This time, the nitrate sediment flux PCA showed more closely clustered scores (Figure 4.22) for all sites, suggesting that the sites behaved more similarly than what was observed with ammonia sediment flux. Nitrate sediment flux behaving similarly between sites is not surprising if this process is fueled by denitrification as this process is robust, optimized by a high variety of bacteria species. The correlation of nitrate sediment flux to amount of *nirS* present could prove this hypothesis. From here, loadings of variables were considered with another PCA biplot in Figure 4.23. The scree plot for the nitrate sediment flux is shown in Figure 4.24. However, again, Unit 2 nitrate sediment fluxes were far enough outside the average for all sites to consider removing from the principal component analysis. Variables were removed again to improve the principal components' proportion of variance. The variables used in the next biplot were the bolded variables displayed in Table 4.13. This time, removing Unit 2 from the principal component analysis (Figure 4.25) showed favorable results. The scree plot for nitrate sediment flux after removing Unit 2 is shown in Figure 4.26.

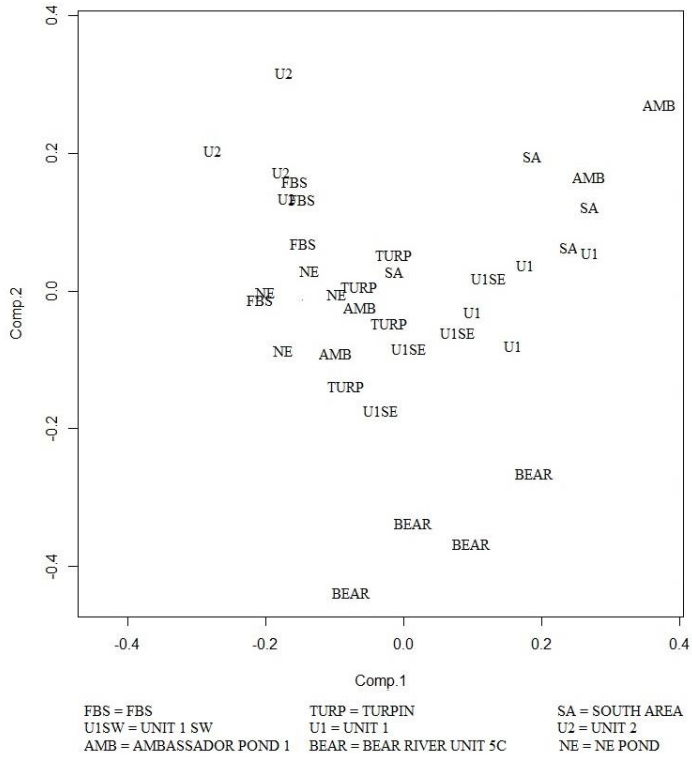

Figure 4.22. Nitrate sediment flux principal component analysis – site scores

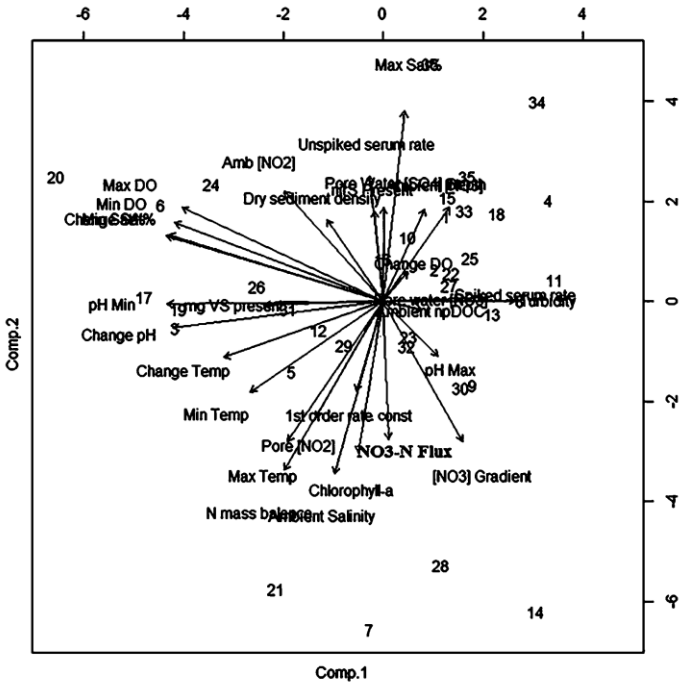

Figure 4.23. Nitrate sediment flux loading biplot - all variables

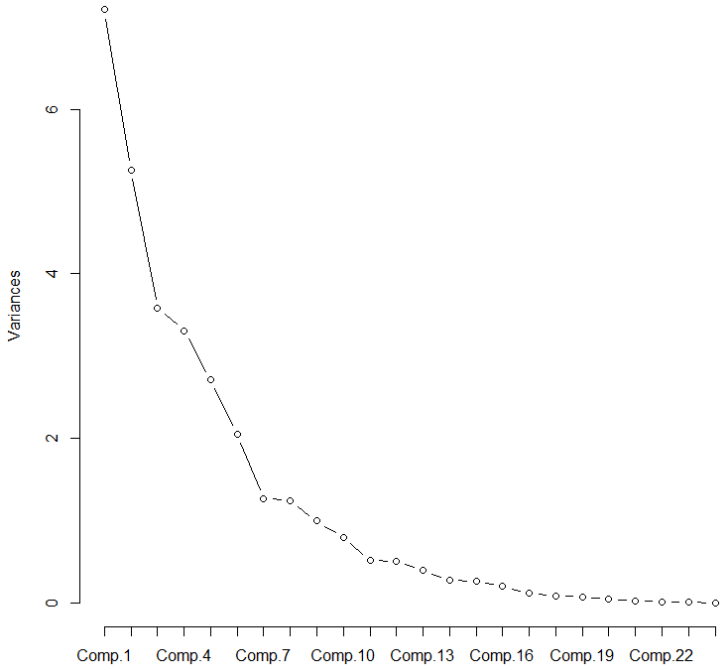

Figure 4.24. Nitrate sediment flux scree plot with all variables

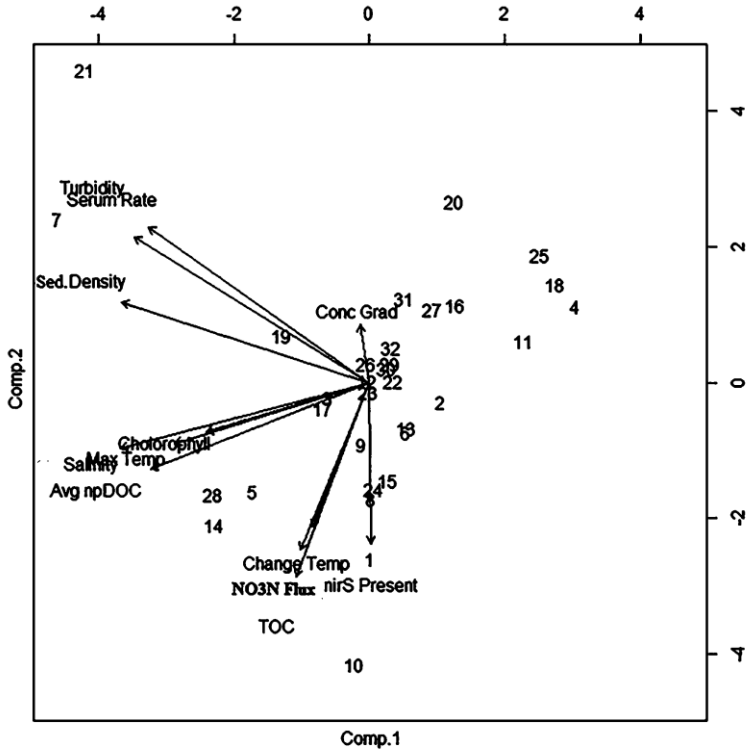

Figure 4.25. Nitrate sediment flux biplot without Unit 2 – select variables

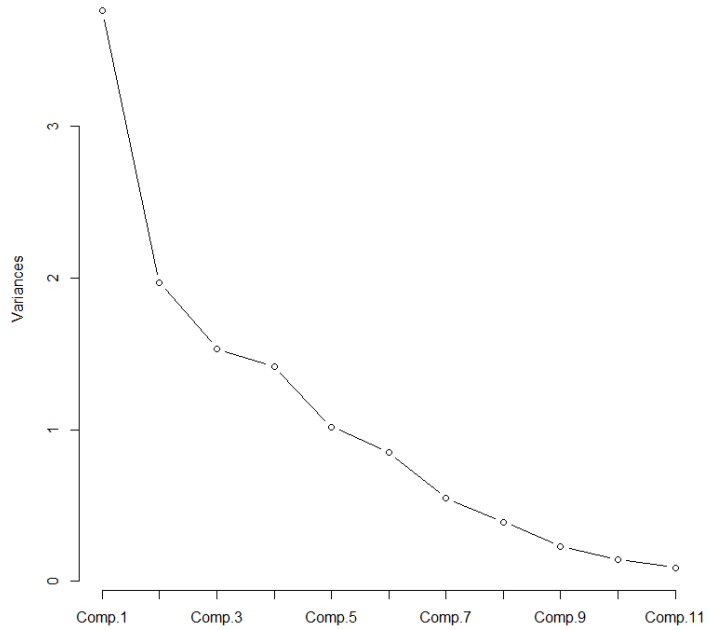

Figure 4.26. Nitrate sediment flux scree plot without Unit 2 – select variables

In this case, the nitrate sediment flux was highly correlated to the amount of *nirS* genes present, TOC amount in soil, and change in temperature of the ambient water. The nitrate gradient between pore water and ambient water was less of a factor in sediment flux than with ammonia. These variables prove that nitrate flux in the sediment is being dictated by bacterial processes, since denitrification is carried out partially by *nirS,* while incorporating carbon. Upon further investigation, the correlation between the TOC of sediment and bacterial control of sediment nitrate flux does not align with the fact that heterotrophic bacteria consume mostly nonpurgable dissolved organic carbon (Metcalf and Eddy, 2003). In this case, the pore water concentration of npDOC was not directly measured; however, TOC of the sediment would have included pore water npDOC – this could be the reason nitrate sediment flux is correlated with both the amount of denitrification bacteria present and TOC of sediment. Last, denitrification and other bacterial kinetics are also highly controlled by temperature – another correlated variable according to Figure 4.26. As for

proportion of variance, it improved from 0.232 and 0.170 to 0.314 and 0.164 for PC1 and PC2, respectively, when variables were removed. While this is a positive change for PC1, the proportion of variance decreased for PC2 when removing variables. Also, the scree plots showed less of a change for nitrate sediment flux than for ammonia sediment flux, suggesting that removing Unit 2 had less of a positive effect on variance for nitrate sediment flux than for ammonia sediment flux. Nevertheless, adjusted PCA biplot for nitrate showed promising results of bacterial metabolic correlation to nitrate sediment flux.

Phosphate Principal Component Analysis

Last, the same method of interpreting principal component analysis biplots for scores and loadings was applied to phosphate sediment flux. The variables used to create these biplots are listed below in Table 4.15; the bolded variables were used to create the loadings biplots – this time, more physical chemistry variables were chosen. The score biplot for phosphate sediment flux is shown in Figure 4.27.

Table 4.15. Phosphate sediment flux PCA variables

Water Physical Characteristics	Ambient Chemical Concentrations	Soil Composition	Phosphorus Speciation
Min DO	PO_4 Conc.	TOC	Total P
Max DO	**Salinity Conc.**	Pore PO_4 Conc.	Loosely Bound P
Δ DO	Average npDOC Conc.	Pore SO_4 Conc.	Iron / Aluminum Bound P
Min DO Sat %	**Chlorophyll-a Conc.**	mg VS Present	Calcium Bound P
Max DO Sat %	**Turbidity**	**Dry Density**	Residual P
Δ DO Sat %		**Wet Density**	
Min pH		Porosity	
Max pH		PO_4 Conc. Gradient	
Δ pH			
Min Temp			
Max Temp			
Δ Temp			
Depth			

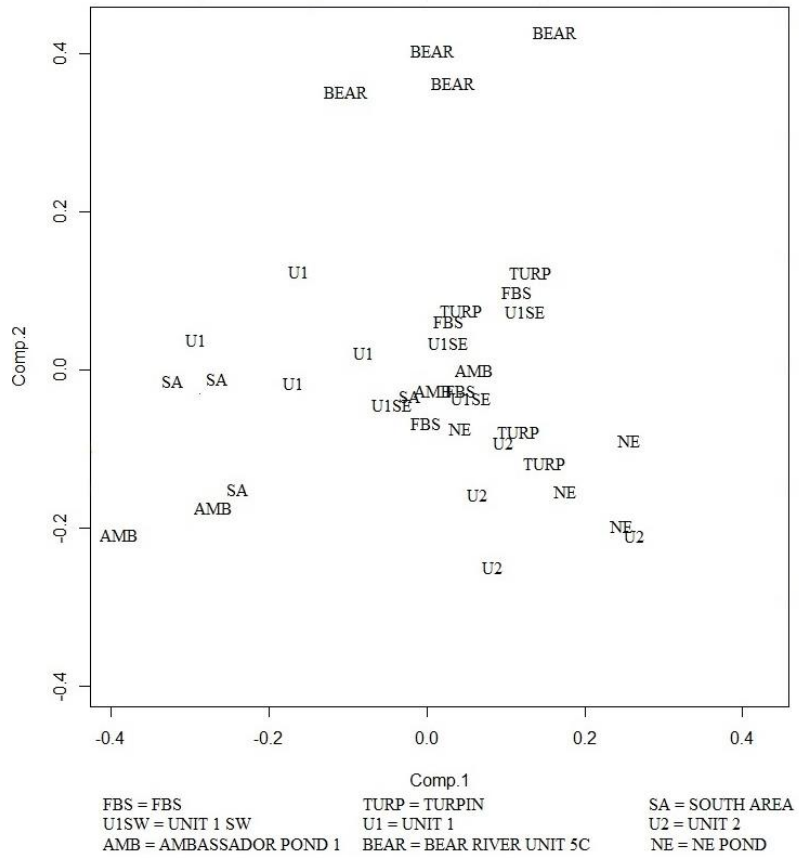

Figure 4.27. Phosphate sediment flux principal component analysis – site scores

The PCA biplot to deduce scores was created using all of the above variables for every site. Again, the scores of each site are based on a complex combination of the 32 variables and show little information about how the sites are related for exactly flux-influencing variables. One interesting trend between all three score biplots reviewed so far is that Bear River Unit 5C consistently shows scores far away from every other site. As this trend was observed in all sediment flux biplots, the variable which may be causing Bear River Unit 5C to be an outlier must be a variable considered for each biplot. After performing a z-test for all variables, Bear River Unit 5C shows turbidity that was very far outside the sample mean. Could turbidity be an influential variable for sediment flux of all

nutrients? Bear River Unit 5C showed nutrient sediment fluxes within the sample mean for all observations, so the high turbidity was assumed to not affect sediment nutrient flux. However, the turbidity variable may cause the score of Bear River Unit 5C to fall outside the average observed scores for every other site. Nevertheless, since the flux values were apparently not altered from turbidity, Bear River Unit 5C was considered for all PCA biplots. All variables and sites were considered for the below phosphate sediment flux biplot in Figure 4.28. The scree plot for Figure 4.28 is shown in Figure 4.29.

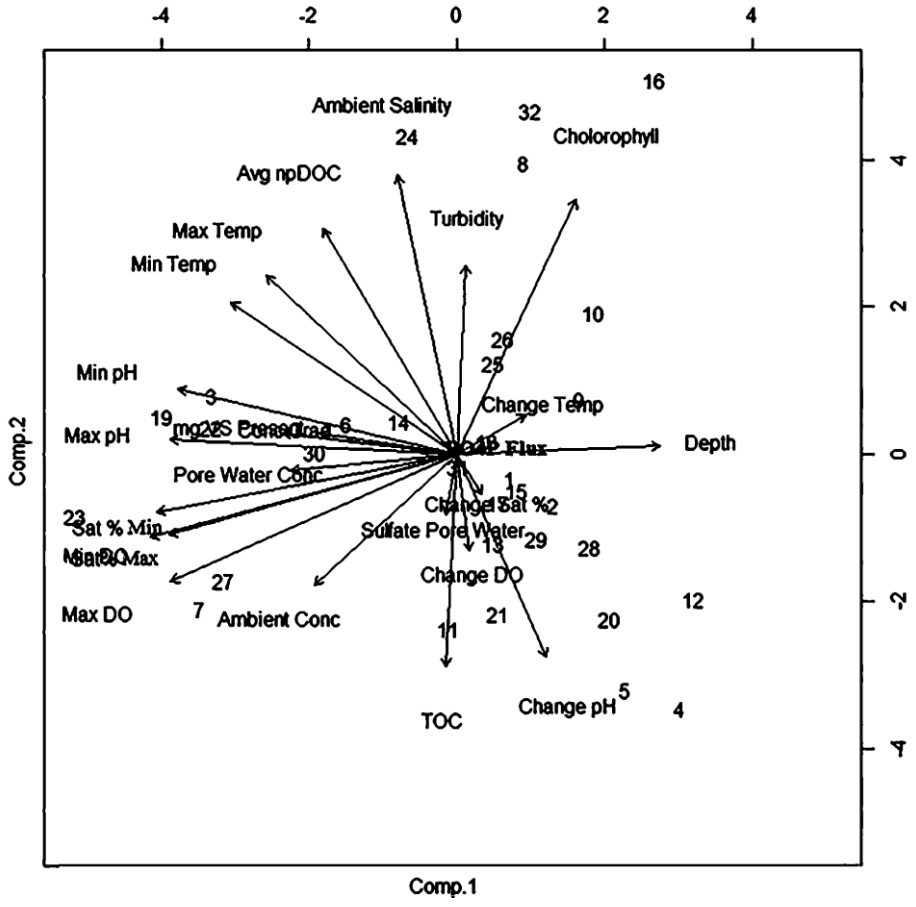

Figure 4.28. Phosphate sediment flux loading biplot - all variables

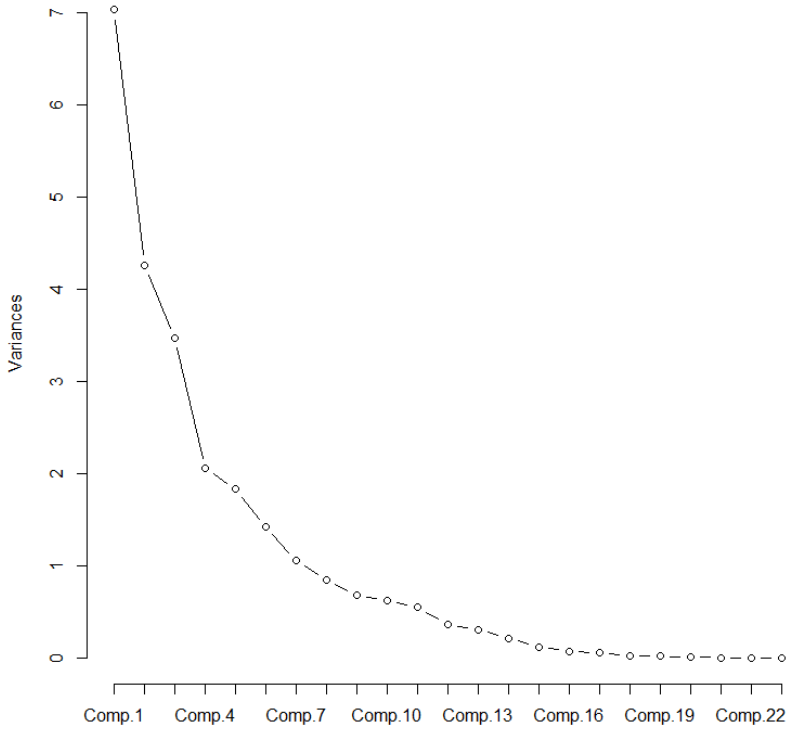

Figure 4.29. Phosphate sediment flux scree plot - all variables

From here, the number of sites was reduced, as were the number of variables, again to show more correlation and to increase each principal component's proportion of variance. This time, a couple more observations were neglected because more than just Unit 2 showed phosphate flux far outside the sample mean. The unspiked early summer sampling of Unit 1 and the unspiked late summer sampling of South Area both showed uncharacteristically large phosphate sediment flux values. Upon further investigation, these sites both showed the largest increase in dissolved oxygen saturation in the ambient water, so this could have caused the large phosphate sediment flux values. The results of removing some variables and sites from the phosphate PCA biplot are shown below in Figure 4.30, followed by the scree plot of this PCA in Figure 4.31.

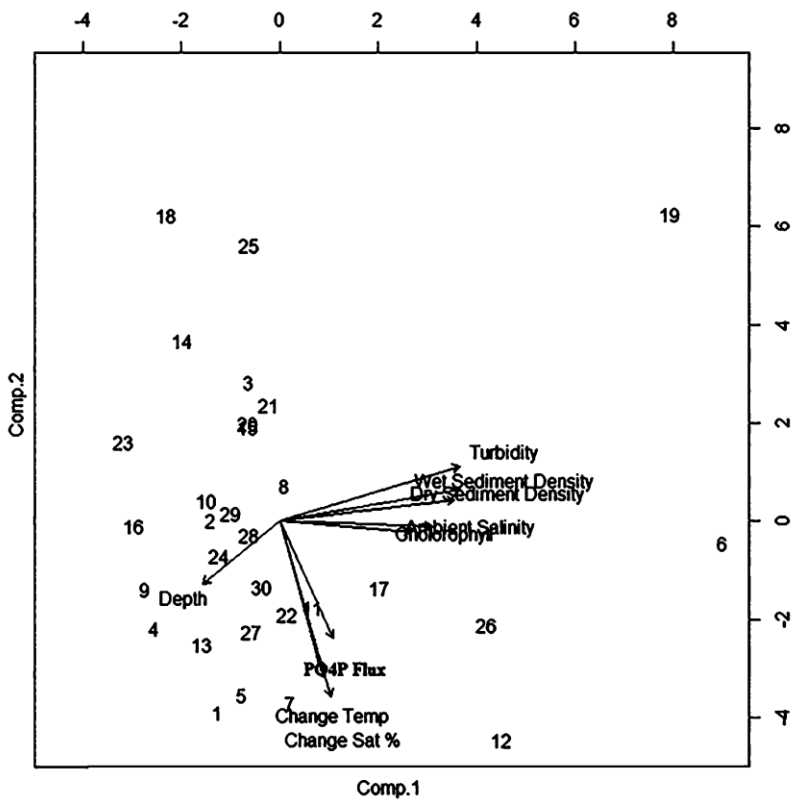

Figure 4.30. Phosphate sediment flux biplot without outlier sites – select variables

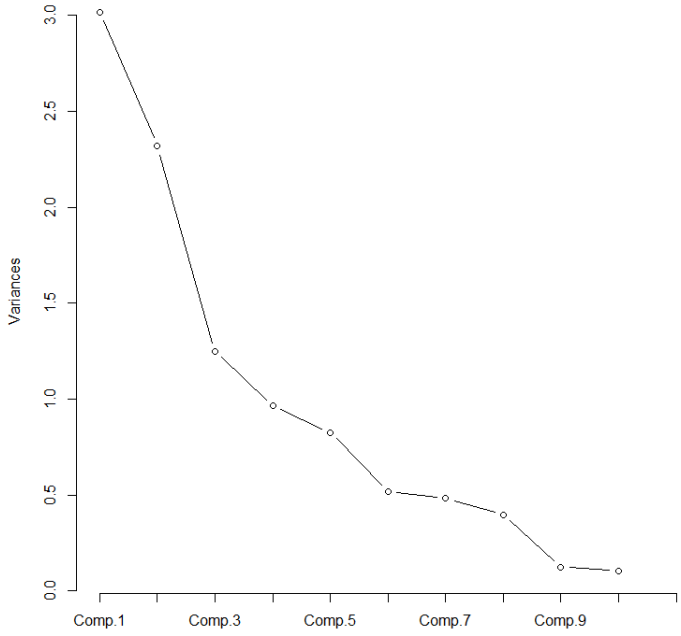

Figure 4.31. Phosphate sediment flux scree plot without outlier sites – select variables

In addition, phosphorus speciation results were compared to phosphate sediment fluxes. Only FBWMA sites were included because speciation results were not measured for Bear River Unit 5C and Ambassador Duck Club Pond #1. No correlation was measured for unspiked phosphate sediment flux, but a strong correlation was found between the amount of iron- and aluminum-bound phosphorus with regards to spiked phosphate sediment flux, as shown in the PCA biplot and scree plot with phosphorus speciation results below. Figure 4.32 shows the biplot results for phosphorus speciation, while Figure 4.33 shows the corresponding scree plot.

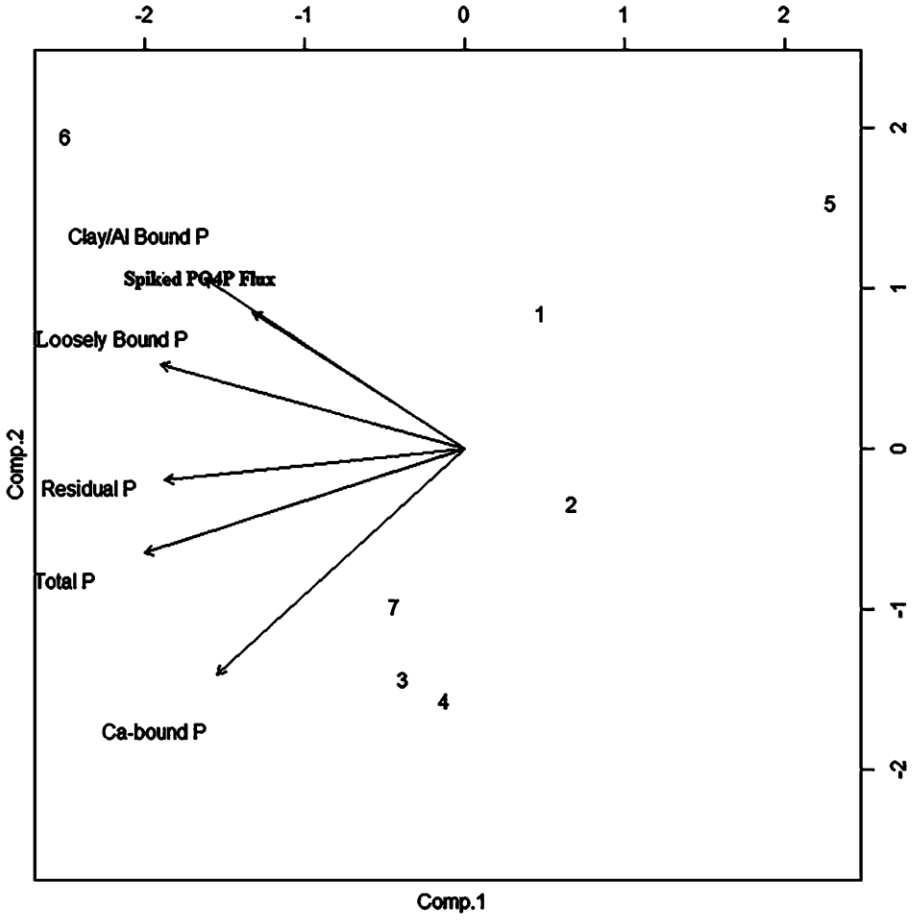

Figure 4.32. Spiked phosphate sediment flux biplot – phosphorus speciation results

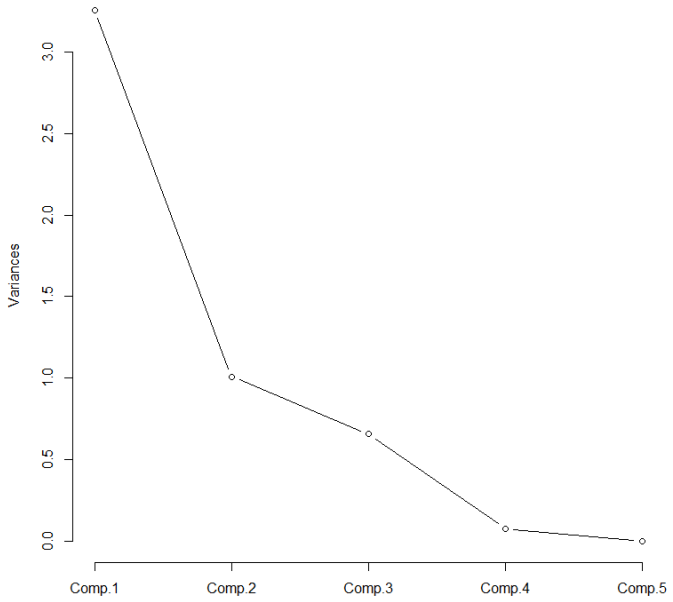

Figure 4.33. Phosphate sediment flux scree plot - speciation results

Based on the PCA for phosphate, phosphate is controlled by some of the expected physical parameters: change in dissolved oxygen saturation and change in temperature of ambient water. These are to be expected as temperature largely influences dissolved oxygen concentrations, and phosphorus release or uptake from sediments as a result of these factors is a well-known environmental phenomenon (Hupfer et al., 2008). However, when tying in the speciation experiment, it becomes obvious than another variable is even more correlated: the concentration of phosphorus attached to clay and aluminum species in the sediment directly corresponds to phosphorus flux under spiked conditions.

The speciation experiment also included iron-bound phosphate with the clay- and aluminum-bound phosphorus measurements. Iron in sediment tends to act as an adsorption site for phosphate. The phosphate is absorbed onto the iron in oxygen-rich conditions as it is redox-controlled, while anoxic conditions force the release of phosphorus from iron (Petticrew et al., 2001). As a result, sites with high amounts of iron-bound phosphorus in the

sediment showed that the sediment was a phosphate source as a function of temperature, and therefore, oxygen saturation in water. This fits the model of iron in sediment acting as adsorption sites for phosphorus—when the sediments at sites have too much phosphorus attached to the iron adsorption sites, the sediment becomes a phosphorus source when it should be a sink. This could have been the case for the spiked phosphate sediment flux experiments – the iron and aluminum in the sediment could have not had enough adsorption sites left for extra phosphate. It should be noted that during ambient conditions, no species of phosphorus was correlated with the sediment flux of phosphate. The correlation of temperature and saturation of dissolved oxygen in the water with ambient phosphate sediment flux and strong correlation of spiked phosphate sediment flux with amount of iron- and aluminum-bound phosphorus in sediment simply suggest that phosphate sediment flux is a redox process. Therefore, while biological processes are correlated with the nitrogen fluxes of sediment, the phosphorus changes are potentially influenced by physical chemistry.

These results show the hypothesis that nitrogen cycling in sediment is more dictated by bacterial processes than other, abiotic processes. The diurnal cycling of phosphorus involving iron and aluminum in the sediment was suggested to be a significant factor in phosphorus sediment flux due to this study, supporting the phosphorus cycling hypothesis.

Conclusions from Task 5

After making some corrections to exclude significant outliers, the advanced statistical technique of PCA was able to support the project's hypothesis of bacterial-controlled sediment processes.

- Unit 2 showed nitrogen flux values which exceeded the other sites' values

enough to justify removal from the PCA. Further investigation showed that the amount of volatile solids in the sediment was also far more than any other sites, indicating higher porosity and thus much higher solute transfer rates.

- Ammonia sediment fluxes showed correlation to *amoA* gene copy amounts, the ammonia concentration gradient between pore water and ambient water, and the amount of ambient nitrite in the water column.
- Nitrate sediment fluxes also showed bacterial contributions as the *nirS* gene copy was correlated to nitrate flux as well as TOC in the sediment and changes in temperature throughout the sampling period.
- Phosphate sediment fluxes displayed more physical and chemical dependency than nitrogen transformations. Correlation to dissolved oxygen saturation changes and temperature changes throughout sampling period to phosphate sediment flux indicate somewhat a dependence of phosphate sediment flux on diurnal processes. The correlation of spiked phosphate sediment flux with iron- and aluminum-bound phosphorus indicates an adsorption reaction happening between phosphorus and sediment which is also theoretically controlled by dissolved oxygen in the ambient water.

CHAPTER 5

CONCLUSION

Understanding the sediment contribution of nutrients to the overlying water quality is paramount in creating a healthier, stable ecosystem in the Farmington Bay Wetlands. The following key points can be concluded from this completed project.

- Ammonia sediment fluxes in the wetlands were more correlated to AOB population size and the concentration gradient between pore and ambient water than other biogeochemical processes. In this way, pore water ammonia concentrations must have been high enough to support AOB activity and solute transport.
- Nitrate sediment fluxes in the wetlands also show correlation to bacterial processes. The gene number of *nirS,* as well as the TOC content of the sediment and ambient water temperature, showed higher correlation to nitrate flux than other variables.
- Phosphate sediment fluxes in the wetlands show correlation to physical properties in the wetlands. Phosphate sediment flux may depend on diurnal changes of dissolved oxygen and temperature in the water column which could release some phosphorus adsorbed to iron and aluminum in the sediment.
- Unit 2 sediments proved to be the largest sink of ammonia and nitrate, possibly as a result of porous sediment enabling greater solute transfer.

- The most common identified AOB in the wetlands was *Nitrosomonas communis*, followed by *Nitrosomonas cryotolerans,* which requires salinity to grow and can survive below-freezing temperatures.
- All sites displayed a net negative nitrogen mass balance during laboratory serum bottle testing.
- The denitrification community contains a high complexity that requires further investigation.

While other studies (White et al., 2003) touched on quantifying nitrification and denitrification in similar wetlands, no information could be found which gives evidence that these bacterial processes are the main driver of nitrogen cycling in the sediment. On a more specific level, information on the identity of the bacterial community in the Farmington Bay wetlands has been limited – this should serve to give better insight into which species of bacteria exist in this area. The techniques used in this study, such as displaying multiple interpretations of nitrification and denitrification serum bottle tests, could be useful for future wetland studies, as well. For example, spiking the serum bottle tests with more nutrients than what was practiced with this study as to achieve a zero-order relationship between nutrient concentration and nitrification or denitrification rate would be a way to observe and calculate the Michaelis-Menten bacterial kinetic constants (Metcalf and Eddy, 2003). Abiotic factors to phosphorus sediment flux, on the other hand, had been identified in other studies, and this study can just be used as further confirmation of those results (Reddy et al., 1999). Nevertheless, this study should answer some questions of sediment contributions to surface water quality at the Farmington Bay wetlands and the role of wetland sediments as a whole.

While some research questions were answered with this study, other questions

emerged that could lead to further understanding of the sediment contributions to the nutrient cycle. The nitrification bacteria analysis helped characterize the species of AOB in the sediment, but the role of ammonia-oxidizing archaea still has not been explained. Potentially, the sediment flux of ammonia could be more correlated to the amount of ammonia-oxidizing archaea than bacteria, as there is often more archaea in an environment than bacteria (Ergunder et al., 2009). As for denitrification, a more detailed analysis of the species of denitrifying bacteria present could be beneficial, as little information about denitrification bacteria was confirmed for this study. Comparing these results to sediment oxygen demand could tie in more information about decomposition to make steps towards creating a nutrient mass balance for the wetlands. Last, more information about the metals composition of the sediment to relate to phosphate sediment flux would confirm the abiotic control of phosphate sediment flux from this study.

As for the multimetric index, a separate sediment health indicator could be added with further research. All scales explored by the index should have a datum against which other wetlands are measured – a pristine wetland which other wetlands can be compared to as an example of target performance. Therefore, constructing a model for healthy wetland sediment would be required before incorporation of sediment into the multimetric index. To create this model, one option would be to observe multiple healthy, undisturbed wetlands and measure the rates of sediment nutrient flux at these sites. From here, sediment nutrient fluxes from other sites can be compared to the unimpaired wetland sediment nutrient flux to create an overall health rating, just like for the other parameters of the multimetric index.

However, sediment contributions need to be supported by tying in an overall mass balance of nutrients in the wetlands. To have a perspective of just how much the sediment contributes to the water quality of these wetlands as compared to SAV or invertebrate

activity, for example, would prompt more focused efforts in sustaining wetland conditions. Methods such as isotope mapping could be effective for creating mass balances, as the ratio of isotopic nitrogen in surface water can be used to predict the sources of nitrogen (Kendall et al., 2012).

Last, it seems that the hypotheses of this study were supported by the observations – nitrogen in sediment is controlled by bacterial processes, phosphorus cycling occurs as a response to abiotic factors, and, at least for nitrogen contaminants, it appears the wetland sediments could be used as a remediation zone, as the sediment was a net nitrogen sink. Meanwhile, it appears that phosphorus cycling occurred to keep the phosphorus at approximately a steady state in the wetlands. Once the overall contribution of sediments to the mass balance of nitrogen and phosphorus in the surface water is determined, the factors presented in this study can be used to help control these sediment nutrient fluxes to foster better surface water quality.

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